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# Use of genetic tagging to estimate abundance and detect spatial patterns of black bears in New Hampshire

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# USE OF GENETIC TAGGING TO ESTIMATE ABUNDANCE AND DETECT SPATIAL PATTERNS OF BLACK BEARS IN NEW HAMPSHIRE

BY

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## ABSTRACT

USE OF GENETIC TAGGING TO ESTIMATE ABUNDANCE
AND DETECT SPATIAL PATTERNS OF BLACK BEARS IN NEW HAMPSHIRE

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University of New Hampshire, December, 2008

Abundance estimates for black bears (*Ursus americanus*) are an important tool for effective management. Recent advancements in DNA technology have enabled genetic tagging mark-recapture population estimates using DNA from hair samples. I conducted a population estimate using genetic tagging in 2 study sites presumed to have different bear densities in northern New Hampshire (Pittsburg and Milan). To test repeatability, I conducted the genetic tagging estimates in 2 consecutive years. I also compared these estimates to those derived from traditional methods used by the New Hampshire Fish and Game Department (NHFG) using hunter harvest and mortality data. I found that the density estimates produced from the genetic tagging methods were consistent in the 2 years, and were similar to those derived from traditional methods. In 2006, the estimated number of bears in Pittsburg (315 $km^2$) was 70, corresponding to a density of 0.16-0.28 (95% CI) bears/$km^2$. In 2007, the Pittsburg (400 $km^2$) estimate was similar: 78 bears with a density of 0.15-0.24 bears/$km^2$. In Milan (440 $km^2$) during 2006, the estimated number of bears was 106 corresponding to a density of 0.13-0.35 bears/$km^2$. The 2007 Milan estimate (371 $km^2$) was similar with 99 bears and a density of 0.19-0.34 bears/$km^2$. While the traditional methods may be appropriate and more cost effective for density

estimation at a regional scale, I found that the genetic tagging methods were able to detect demographic variation at a local scale. In addition to generating population estimates, I used the genetic information to identify population and spatial genetic structure and to determine if landscape features such as roads and rivers caused resistance to gene flow. I tested for population distinction using the program STRUCTURE, $F_{ST}$ values, and a mean relatedness function. I used a Mantel test of isolation by distance and spatial autocorrelation for the spatial analyses. To assess landscape resistance, I used an analysis of mean relatedness between subpopulations divided by landscape features. Through consensus, I found that the 2 study sites were genetically distinct ($F_{ST} = 0.024$, $P = 0.05$). I also found a positive relationship between genetic and geographic distance ($R = 0.13$, $P>0.0001$), and that females showed spatial autocorrelation through 5 km. Regarding landscape resistance to gene flow, I found that the presence of Route 3 in Pittsburg did not cause genetic differentiation between subpopulations on either side of the road, while the Route 16-Androscoggin River corridor in Milan influenced the genetic population structure of females.

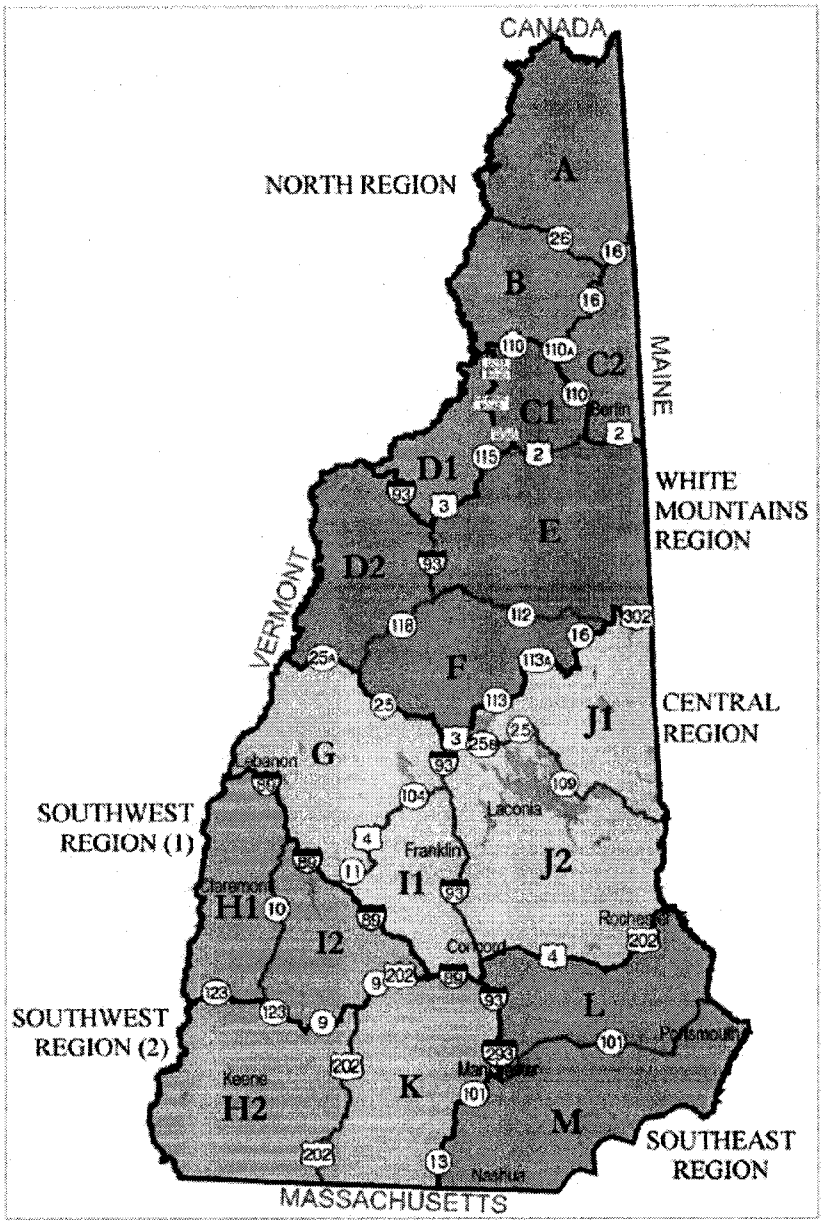
# CHAPTER 1

## INTRODUCTION

Black bear (*Ursus americanus*) management typically falls into 3 categories: conservation, control, and sustained yield. Conservation focuses on managing small or declining populations to increase density; control seeks to stabilize or reduce a population; and in sustained yield, surplus animals are taken without causing population decline (Miller 1989). Sustained yield is the most common management goal in North America, and is the general goal in New Hampshire and more specifically the northern region of New Hampshire including game management units (WMU) A, B, C2, and D1 (NH Big Game Management Plan 2006-2015, Fig. 1).

Managers use a variety of techniques to implement these management strategies. Hunting seasons can be lengthened or shortened, though these controls may have limited effectiveness. For example, in 1979 during a hunting season of just 1 day, 763 black bears were taken in Pennsylvania (Lindzey et al. 1983). Reducing the number of available permits can also help protect against over exploitation. Managers can adjust the hunting season to influence the gender of bears taken. Early spring hunts increase the chance of male take because males leave their dens earlier and roam more freely than females in the early spring (Miller 1989). Or, the season could be opened later in fall and target male bears after pregnant females have denned. Regions can prescribe bag limits such as limiting the number of bears taken from poorly productive areas. Managers can

**Figure 1.** NHFG bear management units (WMUs) in New Hampshire (Timmins 2004).



also control methods of hunting, including restricting the type of weapon, and use of baits and dogs (Miller 1989).

Abundance estimates of black bears are critical for population management. Estimates help managers implement hunting seasons, establish harvest quotas, and monitor population trends. As black bears have low reproductive rates, delayed reproductive maturity, and variable survivorship of young, populations that are overexploited take many years to recover (Miller 1989). Therefore, it is crucial to accurately assess population size to guide scientific management and ensure the stability of a black bear population.

Accurate population estimates can be achieved through several different techniques. Historically, population estimates have been conducted through live capture and mark-recapture techniques, and with radio-telemetry studies that provide the home range sizes for extrapolated density estimates (Rogers 1987). The live capture technique, however, is laborious and expensive, can result in trauma or death of some individuals, and may be biased due to heterogeneity of capture probability (Mills et al. 2000). With the refinement of genetic technology, non-invasive mark-recapture methods have been developed that eliminate the handling of individuals and improve population estimates by increasing the number of observations (Miller et al. 2005).

## Genetic Tagging

Genetic tagging is a non-invasive sampling technique that allows for identification of individuals by collecting hair samples and performing DNA analyses on them to determine unique genetic fingerprints (genotypes; Paetkau and Strobeck 1994, Woods et al. 1999, Bellemain et al. 2005). The genetic tagging method involves 3 steps:

1) collection of hair samples, 2) genetic analysis of hair samples, and 3) mark-recapture population analysis. Hair samples are collected systematically from evenly spaced hair snares throughout the study area over the course of a few weeks. Hair samples are then analyzed and yield a unique genetic profile that "marks" an individual. Subsequent samples either yield identical genotypes and are considered "recaptures" or yield unique genotypes indicative of new individuals. Population size is estimated with mark-recapture algorithms that are based on the probability that a population of a given size would yield the observed capture rate (White et al. 1982).

Genetic tagging offers advantages over conventional censusing methods. It is noninvasive in that it involves no handling of bears, and it requires less field labor because traps are easily constructed and checked once per sampling session. Thus it is more cost effective than daily monitoring of live traps. The laboratory genetic analyses are considered routine, reasonably cheap, and uncomplicated. Genetic tagging studies also have higher capture probabilities, reduced tag loss, and a simple study design that violates fewer assumptions of the mark-recapture models (Mills et al. 2000). Lastly, not only can the genetic data be used to produce a population estimate, it can also yield additional insights about population parameters such as dispersal patterns, paternity, relatedness among individuals, and genetic variability and gene flow (DeYoung and Honeycutt 2005). These data can help managers define management units by assigning individuals to distinct populations and also identifying landscape features that inhibit gene flow.

Interpreting the genetic data using spatial information provides additional insight into the interaction between landscape features and population genetics (Epperson 2003,

Manel et al. 2003). Landscape genetics is a relatively new discipline that uses molecular genetics and spatial statistical tools in conjunction with computer modeling to examine how landscape features influence population structure, specifically in relation to gene flow, genetic drift, and selection. The 2 key processes of landscape genetics are the detection of genetic fragmentation and the correlation of these fragments with landscape and environmental features (e.g., mountains, rivers, temperature, and humidity gradients; Manel et al. 2003). When landscape features restrict gene flow or act as cryptic boundaries of populations, they are called dispersal barriers (Manel et al. 2003, Storfer et al. 2007). This information is critical for managing the genetic diversity of threatened populations and for identifying evolutionary significant management units (Manel et al. 2003). For example, Coulon et al. (2006) used landscape genetics to evaluate whether a fenced highway along a large river limited gene flow in a roe-deer population. They found that the genetic structure of the populations on either side of the highway and river were different, suggesting that the highway and river acted as barriers to gene flow.

Spatial autocorrelation is another statistical tool used in landscape genetics to identify fine-scale genetic patterns. It is used to compare the genetic and geographic distance between individuals to identify the extent of spatial genetic structure in a population (Smouse and Peakall 1999). Positive spatial structure indicates patterns of local relatedness due to restricted dispersal or social organization (Peakall et al. 2003). For widely dispersing animal taxa such as black bears, negative (or random) spatial structure is predicted. However, as female bears are philopatric (Rogers 1987, Elowe and Dodge 1989, Schwartz and Franzmann 1992, Onorato et al. 2004) spatial genetic structure is promoted. Peakall et al. (2003) used this technique to identify positive spatial

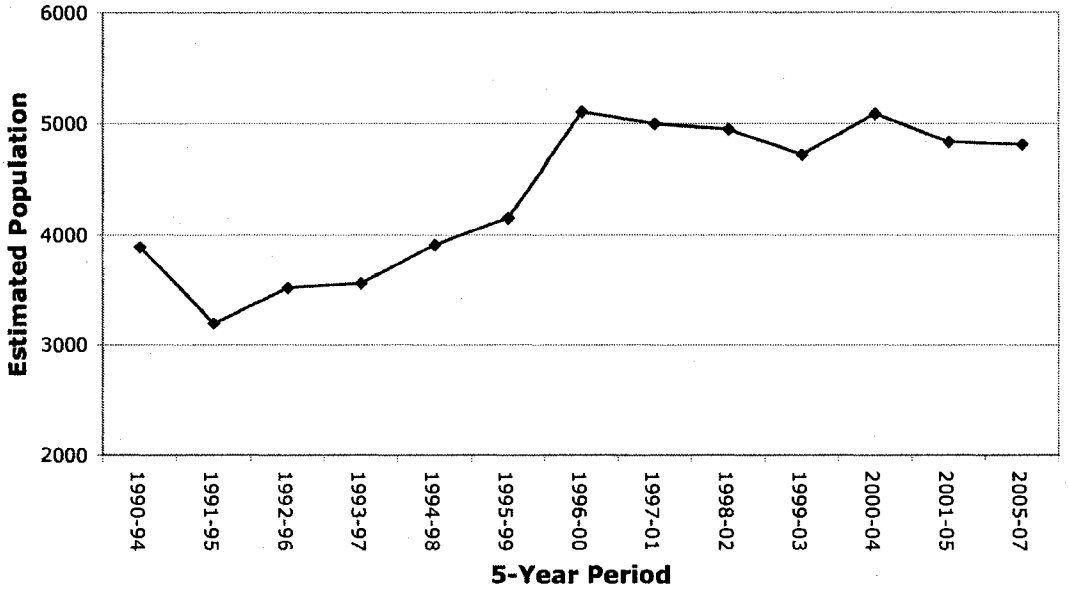
genetic structure in bush rats (*Rattus fuscipes*) through 600 m, indicating that adjacent bush rats within this distance are more genetically similar than distant individuals.

## New Hampshire Black Bear Population and Management

Bear populations in New Hampshire have recovered from record low populations in the mid-1800s and are currently at an all-time high. The elimination of the bounty system in 1955, land-use changes such as farm abandonment, and the implementation of a regulated bear harvest all have encouraged population recovery (Timmins 2004). As the population increased, so did interest in hunting black bears. Black bears were declared a big game species in New Hampshire in 1983, and the New Hampshire Fish and Game Department (NHFG) was granted the authority to regulate the bear harvest in 1988. Hunters have been required to purchase bear hunting permits since 1990 and the number of sales has increased from 4000 in 1990 to about 16,000 in 2003 (Timmins 2004).

Hunters pursue bears only during fall using bait, hounds, and still-hunting techniques. There is a limit of 1 bear per hunter per year, and since 1996 the annual bear harvest has averaged 423 bears. Variation in annual harvest is mostly related to the availability of mast, though hunter effort, season length and timing, and bear population levels also play a role. Harvest typically increases in years with low mast production. As bears move to search for food, often in agricultural areas, their visibility and vulnerability to hunters increase (Timmins 2004). Average harvest rates in New Hampshire are twice as high for males (28%) compared to females (13%). Estimates of the NHFG indicate an increasing bear population since 1990 (Fig. 2). Since 2000, the population has remained

**Figure 2.** Estimated average New Hampshire statewide black bear population, 1990-2007. Population estimates are based on 5-year periods of age and sex mortality data and 3-year periods of deer hunter observation rates (A. Timmins, NH Bear Project Leader, unpublished data).



relatively stable at about 4,800 bears in 2007 (A.Timmins, NH Black Bear Project Leader, unpublished data).

Statewide bear management goals are to maintain the current population, encourage a southward range expansion, and reduce density in the White Mountain region. The specific goal in the northern region of New Hampshire is to maintain the current bear density through sustainable yield (NH Big Game Management Plan 2006-2015). Based on habitat availability alone, New Hampshire could support higher bear densities than already achieved. Bear densities, however, need to be balanced with human tolerance (Timmins 2004). Increased bear densities appear to result in more human-bear conflicts and higher bear mortality, either from bears dispatched due to nuisance behavior, or road-induced mortality. Human population growth results in habitat loss and is likely the major limiting factor to future bear populations.

The New Hampshire bear population is estimated currently from an age and sex analysis of harvest and non-harvest mortality data combined with bear survey data by deer hunters. The Paloheimo and Fraser (1981) model is used to estimate harvest rates, which can be applied to total mortality to back calculate a statewide population estimate. Deer hunter observation rates are then used as an indicator of regional bear densities and help translate statewide population estimates into regional bear densities (Timmins 2004).

While estimating population size using hunter-harvest and mortality data is less costly than live capture or telemetry studies, there is concern that it is not the most reliable index (Miller 1989, Kane and Litvaitis 1992, Koehler and Pierce 2005). Due to small sample size, hunter-harvest and mortality data provide broad scale population estimates that cannot detect local variation in bear density (Miller 1989). Koehler and

Pierce (2005) caution that harvest statistics do not always accurately assess total mortality because hunter-wounding losses and poaching often go unreported. There is also a concern that the hunter-harvest and mortality data do not accurately represent the demographics of the true population. Kane and Litvaitis (1992) compared the age and sex composition of hunted bears in northern New Hampshire with live-captured bears and found discrepancies in age structure and sex ratios of the 2 samples, suggesting that neither index should be used in isolation to estimate population size. In addition to biases in hunter harvest rates, bear observation by deer hunters may be biased due to variation in detectability in different regions of the state.

In 2003, a pilot study was initiated to determine the feasibility of using genetic tagging in conjunction with mark-recapture techniques to estimate the black bear population in the Connecticut Lakes Headwaters Forest (Forest) in the town of Pittsburg, NH (Kovach and Pekins 2004). Based on the success of that pilot study, the current study was developed with an improved study design to compare population estimates derived from the genetic tagging technique to those derived from harvest and mortality data used by the NHFG.

## Objectives

The general objectives of this study were twofold: to use a genetic tagging mark-recapture approach to conduct a population estimate in 2 consecutive years for 2 study sites presumed to have different bear densities, and to determine if population genetic structure was influenced by spatial and landscape features. More specifically, for the population estimate I aimed to: 1) estimate the density of the black bear population in the Pittsburg and Milan study sites; 2) determine the sex ratio of the 2 populations; and 3)

compare the population density estimates with existing estimates derived from hunter survey and harvest statistics. My specific goals for describing spatial genetic structure were to: 1) determine if the 2 study sites were genetically distinct; 2) investigate the relationship between genetic and geographic distance on both a broad and fine scale; and 3) determine if landscape features such as Route 3 in Pittsburg and the Route 16-Androscoggin River corridor in Milan limited gene flow. I predicted the 2 study sites were distinct populations linked by minimal gene flow, because the philopatric social organization of black bears in conjunction with the distance between the study sites (43 km; 27 miles) should generate genetic differentiation. I also predicted that individual relatedness decreased with distance due to the presence of female philopatry. Finally, based on evidence that roads inhibit gene flow in brown (*Ursus arctos*) and black bears (Thompson 2003, Proctor et al. 2005), I predicted that Route 3 and the Route 16-Androscoggin River corridor were features that restricted gene flow in black bears.

# CHAPTER 2

# METHODS

## Study Sites

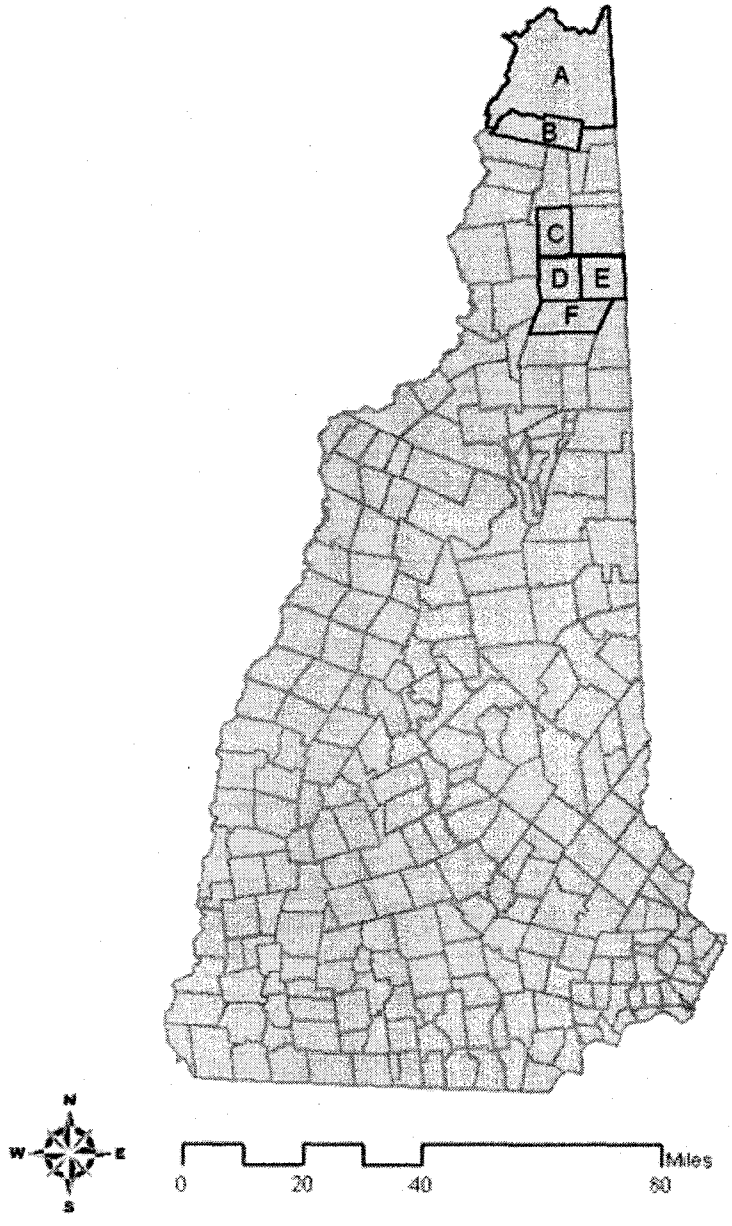
### Pittsburg

The Pittsburg study site (Pittsburg) contained the towns of Pittsburg and Clarksville and was located in the northern part of Coos County in northern New Hampshire (Fig. 3). The study area is a part of the 146,400-acre Forest that includes much of the northern tip of New Hampshire and represents the largest unbroken tract of privately owned forestland in the state.

The Connecticut Lakes region is part of the Northern Appalachian/Acadian Ecoregion and is characterized as predominately forested and hilly, but not dominated by high mountains (CLTC 2004). Hardwoods such as sugar (*Acer saccharum*) and red maple (*Acer rubrum*), yellow birch (*Betula alleghaniensis*), and American beech (*Fagus grandifolia*) dominate the forest type with a tendency toward higher concentrations of softwood such as red spruce (*Picea rubens*) and balsam fir (*Abies balsamea*) in areas above 760 m (2500 ft) and in colder, wetter lowlands and stream valleys.

This land has broad ecological, recreational, and commercial value. The numerous wetlands, bogs, ponds, and lakes support waterfowl and wetland-dependent species, while the upland forest habitats support many species of wildlife (CLTC 2004). Moose (*Alces alces*), white-tailed deer (*Odocoileus virginianus*), black bear (*Ursus americanus)*, ruffed grouse (*Bonasa umbellus*), snowshoe hare (*Lepus americanus*), and

**Figure 3.** Study site locations for population estimates using genetic tagging in northern New Hampshire. The Pittsburg study site consisted of towns: A. Pittsburg and B. Clarksville. The Milan study site consisted of towns: C. Millsfield, D. Dummer, E. Cambridge, and F. Milan.



woodcock (*Scolopax minor*) are all common, and the land is frequently used for hunting, fishing, and wildlife viewing (CLTC 2004).

The Forest has a long history as a large industrial forest and has had several owners and management styles. Due to numerous natural disasters, including a spruce budworm (*Choristoneura fumiferana*) attack in 1973 and a devastating ice storm in 1998, management practices included an extensive amount of salvage clear-cutting. In July of 2001, International Paper put all of their 171,326-acres on the market (Staats and Kelly 2006). Recognizing the importance of this immense natural resource for the local economy, timber industry, conservation, and recreation, several interested parties came together with the goal of permanently protecting the land. With broad state cooperation, the Trust for Public Lands (Trust) was able to purchase the property. The Nature Conservancy acquired 25,000 acres of the land and deeded the land to the NHFG for the creation of a protected natural area that is managed for wildlife habitat and biodiversity (Staats and Kelly 2006).

The Trust then developed a framework to protect the ecological, social, economic, and historical values of the remaining 146,400-acres. The plan included a recreational strategy, a road maintenance agreement, and a working forest conservation easement (CLTC 2004). Today the working forest is operated under the name of the Connecticut Lakes Timber Company (CLTC) and the entire forest is subject to the terms of a conservation easement held by the state of New Hampshire. With the completion of easement purchase efforts in the Forest, and the greater availability of public access, the NHFG expects an increase in bear hunting as well as a push to liberalize the methods by which bears are harvested, specifically bait hunting. These expectations have led the

NHFG to focus attention on management of the region by assessing baseline bear populations in order to monitor for future changes.

**Milan**

The greater Milan area (Milan) was located in the southeastern part of Coos County in the Mahoosuc-Rangeley Lake region of northern New Hampshire, within the Androscoggin River watershed in the towns of Milan, Dummer, Millsfield, and Cambridge (Fig. 3). The terrain is rolling to slightly mountainous except immediately adjacent to the Androscoggin River flood plain. The study area consists primarily of commercial forestland divided into many private ownerships, with small areas of cultivated land adjacent to the Androscoggin River. Forested habitat of a variety of forest types makes up most of the region. Approximately 1/3 of the region is deciduous northern hardwood forests that consist of a mix of yellow birch, beech, and sugar maple. The second third is made up of spruce-fir forest found at nutrient-poor or poorly drained sites at lower elevations. Mixed hardwood-coniferous forest makes up the final third of the region with species composition ranging from the northern hardwoods (beech, maple, and yellow birch) to spruce-fir (Degraaff et al. 1992, Sperduto and Nichols 2004). Commercial timber harvesting or less common natural disturbances create early successional stands comprised mostly of quaking aspen (*Populus tremuloides*) and pin cherry (*Prunus pensylvanica*), with an abundant shrub layer of raspberry (*Rubus spp.*; Scarpitti 2006). Recreation such as hunting, trapping, fishing, and camping/hiking is common throughout the area.

## Sampling Methods for Population Estimation

### Trap Sites

Results of the pilot study (Kovach and Pekins 2004) showed a heavily male-biased (about 3:1) sex ratio of captured bears, suggesting that the trap density (1 trap per 13 $km^2$) was too low to effectively capture less mobile females. Therefore, in an attempt to increase female captures, I reduced the cell area and increased trap density to 3 traps per female spring/summer home range (20 square kilometers; Meddleton and Litvaitis 1990), or 1 trap per 5.2 $km^2$. I established hair traps in a systematic grid design (Woods et al. 1999) by dividing each study site into 5.2 square kilometer (2 square mile) cells and constructing 1 hair trap within each cell (Fig. 4 and 5). Trap locations were initially identified on maps based on road access and distance from adjacent traps. Most traps were placed in contiguous cells at least 1.6 kilometers (1 mile) apart, excluding inaccessible cells. Fifty traps were established in Pittsburg and 51 in Milan.

### Hair Traps

A hair trap consisted of a single barbed wire strand approximately 20 m long wrapped around 4 trees, 40 cm above the ground (Woods et al. 1999; Fig. 6). I baited traps weekly with Ultimate Bear Lure® (Wildlife Research Center, Ramsey, MN) by saturating a square cotton fabric hanging from 2 trees, 3 meters (10 feet) above the ground. One quart of steamed flaked corn was placed on the ground in the middle of each trap to help attract bears. Bears attracted by the bait/scent crawled underneath (or over) the barbed wire that snagged their hair in the barbs. All hair from each barb was collected, categorized by trap session, trap number, and approximate number of hairs, and placed in small paper envelopes. The barbs were then flame-sterilized using lighters to

**Figure 4.** Locations of hair traps (+) in Pittsburg, New Hampshire, summer 2006-2007.

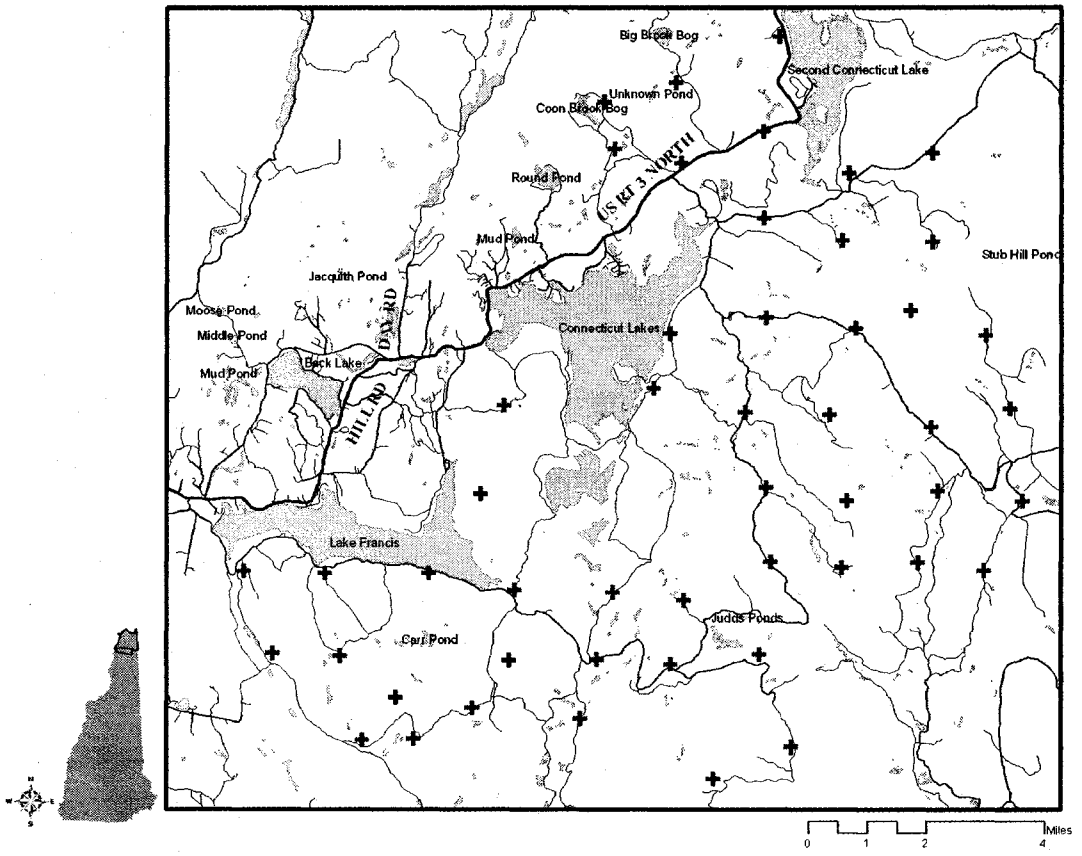


**Figure 5.** Locations of hair traps (+) in Milan, New Hampshire, summer 2006-2007.

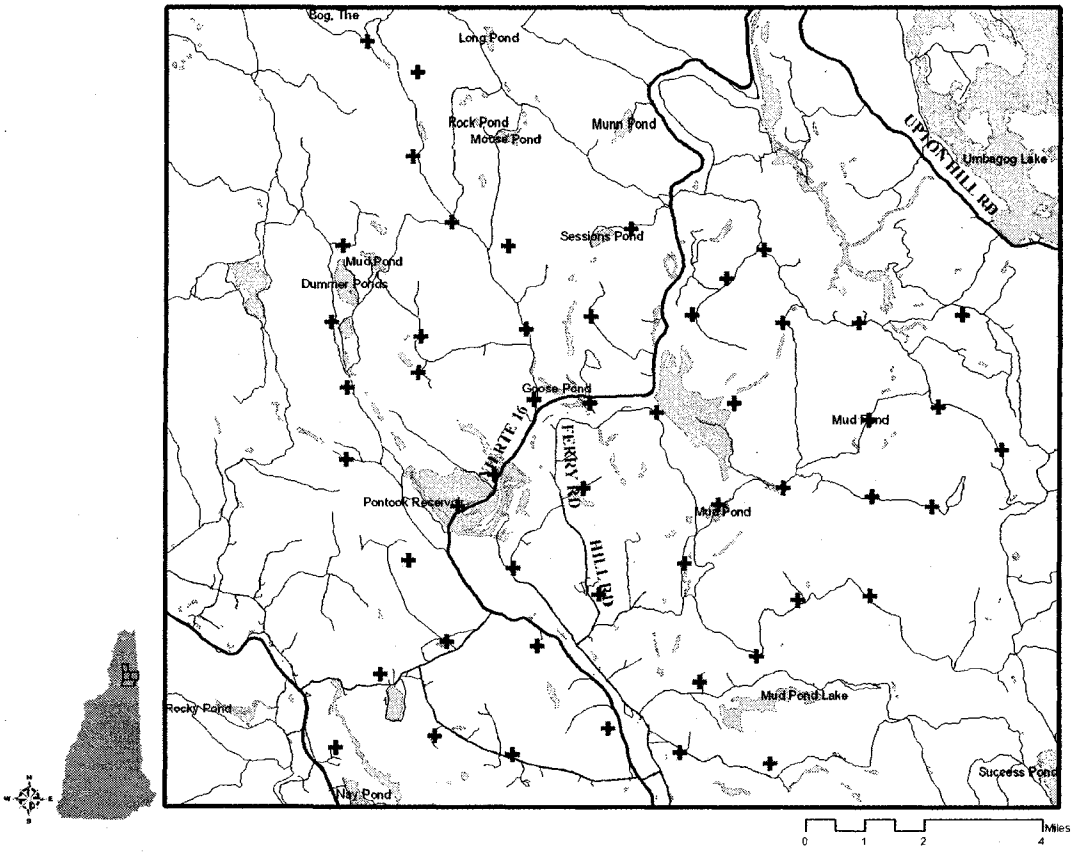
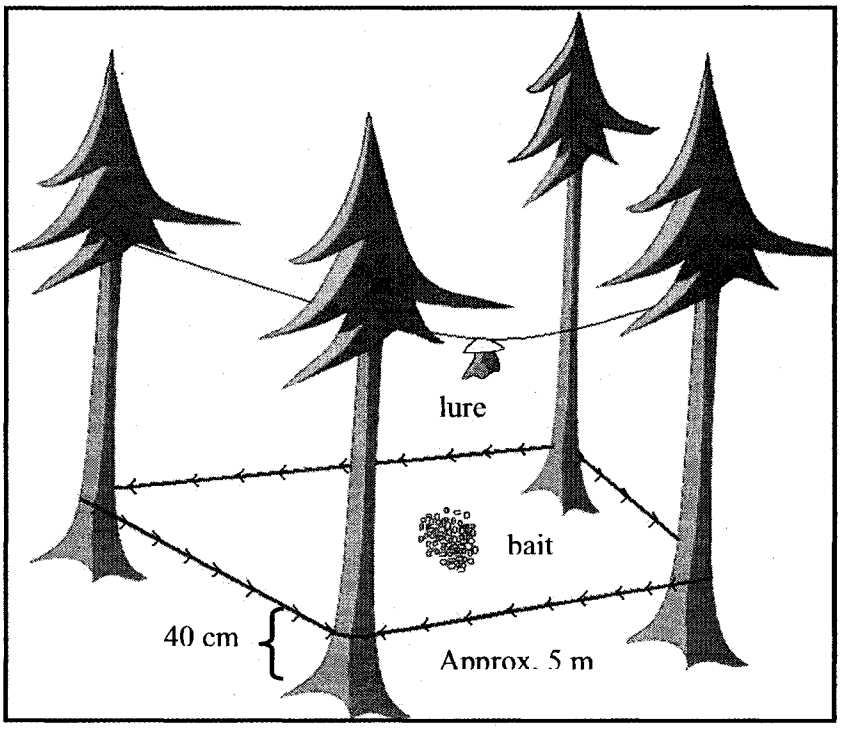


**Figure 6.** Design of traps used to snag bear hair for DNA extraction and subsequent population estimation in northern New Hampshire, 2006 and 2007. One quart of steamed flaked corn was used as bait, and Ultimate Bear Lure® (Wildlife Research Center, Ramsey, MN) was used to attract bears.



prevent contamination. All samples were kept dry and frozen within a day of sampling. Traps were checked every 7 days during 8 consecutive weekly trapping sessions in May-July. Hair traps were sampled during the summers of 2006 and 2007 using replicated methods in both study areas.

**Sub-Sampling**

Ideal hair samples consisted of at least 8 guard hairs with visible follicles because they yield sufficient DNA for genotyping (Goossens et al. 1998, Kovach and Pekins 2004). Due to the high sample yield, a subset of the total samples was genotyped from each study site. Sub-sampling consisted of selecting a single sample of ≥8 hairs from each trap line (the 4 perimeter strands of barbed wire of a single trap). More than 1 hair sample from each trap was included in the sub-sampling regime to screen for multiple individuals captured in a trap session. Because some traps did not have samples on every line, this resulted in 0-4 samples per trap per session.

## DNA Extraction and Amplification

DNA was extracted from the hair samples using a QIAGEN QIAamp DNeasy Blood and Tissue® kit (Valencia, CA) with the slight procedural modification of adding dithiothreitol (DTT) to the lysis buffer to break down the di-sulfide bonds found in hair proteins. Genotyping was performed with the following 6 highly variable microsatellite markers: G1A, G10B, G10C, G10L, G1D, G10X (Paetkau and Strobeck 1994). Gender identification was performed with a Y chromosome marker (SRY gene fragment) that amplifies only in males. To ensure correct gender identification, 2 different SRY fragments were amplified and analyzed independently. The primers used were SRY41F and SRY121R (Taberlet et al. 1993) and a modified SRY that yields a smaller sized

fragment (Bellemain and Taberlet 2004). Extracted DNA was eluted with 75 μL of AE Buffer. Genetic samples were amplified in a 25 μL polymerase chain reaction (PCR) with the following conditions: 1x Taq buffer (Promega), 0.2 mg/mL Bovine Serum Albumin (BSA), 0.2 mM each deoxynucleotide-triphosphate (dNTPs,), 2 mM $MgCl_2$, 0.16-0.0.36 μM of each primer, 1 unit of Taq polymerase, and 5 μL of template DNA. Amplification was performed in an Eppendorf mastercycler (Eppendorf, Westbury, NY) and consisted of 35 cycles of 30 s at 94 °C, 45 s at 58 °C, and 1 min at 72 °C, all preceded by 4 min at 94 °C and followed by 10 min at 72 °C (Kovach and Pekins 2004).

## Genotyping Analysis

Amplified products were analyzed with fluorescent dye-labeled primers (FAM, HEX, OR NED) and electrophoresed with an automated DNA capillary sequencer (ABI 3130). I analyzed several loci at the same time (multiplexing) by amplifying several primers in the same reaction. I developed 2 sets of primers for multiplexing: the first set included G1A, G10B, G10C, and short SRY; the second set included G10L, G1D, G10X, and SRY. In the second set, G10X was amplified separately and later mixed with the amplified product of the other primers (ratio of 2:1) for genotyping analysis. The program PEAKSCANNER was used to aid in the manual scoring of genotypes.

## Discrimination of Individuals

To demonstrate the strength of the genetic data used in population estimation, it is important to assess the power of the genetic markers for individual identification. The probability of identity ($P_I$) is a measure of how powerful the genetic markers are and can be defined as the probability that a random individual in the population has the same genotype as another individual in the population (Taberlet and Luikart 1999). $P_I$ is

calculated by first using allele frequencies to calculate the probability of 2 individuals having the same genotype at each locus. Then, the product rule is used to multiply the probabilities across all loci to obtain a multi-locus $P_I$ (Taberlet and Luikart 1999). A low probability of identity indicates a low likelihood of different individuals sharing the same genotypes and therefore a high confidence in the detection of 2 unique individuals. In wildlife populations, individuals are usually not randomly located in space, but rather may occur in family groups such as a mother and her cubs. To account for sampling close relatives with similar genotypes, a more stringent probability statistic is needed for estimating the power of individual identification (Woods et al. 1999). To this end, a statistic that estimates the probability of identity for siblings ($P_{Isib}$) was developed; $P_{Isib}$ is the probability that 2 siblings in the population have the same genotype. The $P_{Isib}$ value should be larger than the $P_I$ value due to an increased probability that a sibling will have similar alleles. The program DROPOUT was used to determine the $P_I$ and $P_{Isib}$ values across loci.

Woods et al. (1999) described a $P_{sib}$ "match" test that estimates the probability of identity for each individual genotype (as opposed to $P_I$ and $P_{Isib}$ which estimate the probability of identity over all genotypes for the population data set as a whole). The test determines the probability that a given individual has the same observed genotype as its sibling. While $P_I$ and $P_{Isib}$ describe the power of the genetic markers, the $P_{sib}$ match test sets the identity criterion for each individual. Allele frequencies can influence the $P_{sib}$ value because individuals with common alleles are more likely to match other genotypes in the population and thus may not meet the criterion for the detection of unique individuals (Woods et al. 1999). Following Woods et al. (1999), the criterion for

accepting unique genotypes was set to $P<0.05$ (Mowat and Strobeck 2000, Boerson et al. 2003).

With non-invasive DNA sampling, DNA is often in low quantity and of poor quality and as a result genotyping error can occur. Genotyping error is the difference between the true genotype and the observed genotype of an individual, and it can be caused by low or poor quality template DNA that results in enzyme slippage errors in the PCR. Examples of genotyping error include the failure of 1 allele to amplify (allelic dropout), which leads to a homozygote score when the individual is actually a heterozygote, and misprinting when artifactual or erroneous amplification products are generated and mistakenly read as true alleles (known as false alleles; Hoffman and Amos 2005). Non-negligible human error can also occur in scoring the genotypes (Bonin et al. 2004) and precautions must be taken to ensure accurate results.

Over time several error-checking methods have been suggested. Taberlet et al. (1996) suggested a multiple tubes approach, whereby each sample is replicated up to 7 times; but this can become cost prohibitive and its necessity has been questioned (Paetkau 2003, Schwartz et al. 2006). In response to these limitations, Paetkau (2003) proposed a method whereby all samples are initially genotyped once, then all pairwise samples are compared and those with the same genotype at all but 1 or 2 loci, called a single or double mismatch, are reanalyzed. This approach dramatically reduces the need for reanalysis and is more cost effective, though it has been criticized for its lack of a formal test for efficacy of error removal (McKelvey and Schwartz 2004). In an effort to efficiently minimize genotyping error, I used a compromise protocol, by which I repeated

analysis of all homozygote or otherwise suspect alleles (up to 4 times) until I was confident of the genotype, and in addition, I also checked for mismatches.

For microsatellites with dinucleotide repeat motifs, as used in this study, the genotypes are expected to score in multiples of 2. However, when using gel electrophoresis to genotype individuals, amplified microsatellite fragments rarely size at such strict intervals. To account for this variation, during the first sampling season I used the program FLEXIBIN (Amos et al. 2007) to convert raw allele lengths to allele classes (bins). In the second sampling season, I manually assigned alleles to bins, as it was more consistent and improved error checking.

Once all the genotypes were scored, I identified unique individuals by sorting the data in Microsoft EXCEL and searching for matching genotypes. Samples with matching genotypes were considered to be the same individual. I then used the program DROPOUT (McKelvey and Schwartz 2005) to identify the individuals that had identical genotypes at all but 1 or 2 loci (single or double mismatch). This yielded a list of individuals that had similar genotypes, and I reviewed the raw data to ensure that each was unique. Due to the large number of samples genotyped, many individuals were genotyped more than once, which helped to match inconsistent genotypes. For example, in sorting the data by trap session and trap, I scored multiple genotypes from the same individual and corrected genotyping errors. DROPOUT was then used to produce a capture history for each individual. Individual bears were identified by their genotype and gender (using the SRY marker) and the sex ratio of each population was determined by counting the number of males and females.

Estimating the genotyping error rate is useful in the calculation of error from

allelic dropout or mis-scoring, and it reveals the number of discrepancies in the data set if no procedures were in place to correct for this error. With the replications built into my procedure, genotyping error is minimized to negligible levels. However, it is important to declare the genotyping error rate for the purpose of procedural evaluation (Taberlet and Luikart 1999, Bonin et al. 2004, Hoffman and Amos 2005, Pompanon et al. 2005). To estimate the genotyping error rate, I randomly selected 8% (N = 92) of the total samples (N = 1111) and re-analyzed them. I then counted the number of discrepancies between the reference and the re-sampled genotypes to find an error rate per allele, locus, and multilocus genotype.

## Mark-Recapture Population Estimation

Mark-recapture or capture-recapture is a technique that is widely used in population estimation. Ecologists generally sample an area for "counts" of a species to estimate the size of a population. These counts alone are not informative because they represent only the sampling fraction of the larger population. Capture-recapture models were developed to use several count sessions to estimate the sampling fraction and extrapolate for a population estimate (White et al. 1982). Capture-recapture models can be developed for an open (animals enter and leave) or closed population (population stays the same). Most closed capture-recapture models have 3 important assumptions (Seber 1973): 1) the population is closed, 2) animals do not lose their marks during an experiment, and 3) all marks are noted and recorded correctly at each sampling occasion.

The earliest capture-recapture model is the Lincoln-Peterson method that is based on the ratio of marked individuals within a population. It involves catching an initial sample of animals ($n_1$), applying marks to each animal, and then releasing them back into

the population. Later, another sample of animals is captured ($n_2$) and the number of animals that are marked in this sample are recorded ($m_2$; i.e., some animals are already marked and some are unmarked). The sampling fraction is then $n_1/N$ where N is the true number of animals in the population. If the assumptions of the model are correct, then the proportion of marked animals in the second sample can be used to estimate the size of the population (Seber 1973). The equation is:

$$m_2 / n_2 = n_1 / N,$$

and to estimate the population size the equation is:

$$N = n_1 n_2 / m_2$$

More sophisticated capture-recapture models have been built upon the Lincoln-Peterson method. The Lincoln-Peterson method only utilizes 2 sampling sessions, and advances in statistical programs have enabled researchers to create models that include more sampling sessions as well as reflect the dynamic nature of natural animal populations and behavior. Biostatisticians have refined the estimator to reflect different assumptions and reduce bias (Nichols 1992).

Due to the short duration of the study period, in which bear biology suggests there is no birth and limited death, a closed population model was used to estimate abundance. A capture history was constructed for each individual and used in the computer program CAPTURE (Otis et al. 1978). CAPTURE works by selecting a model that best describes sources of variation in the data. Three such models predict variation in capture probability by time, behavioral response, and heterogeneity. Factors such as weather and temperature can vary over time, bears may have behavioral responses such as trap avoidance or trap fascination, and individual responses due to differences in age, sex,

dominance, or activity fall under the heterogeneity model (White et al. 1978). These 3 sources of variation and their combinations make up the 10 models in the program CAPTURE. Ultimately, each model is tested on the data set and the simplest, best-fitting model is applied to the data.

To test for closure, I used the program CLOSETEST (Stanley and Burnham 1999), which performs 2 specialized tests, 1 based on Otis et al. (1978) and a second based on Stanley and Burnham (1999). The Otis et al. (1978) test is unaffected by the presence of heterogeneity, but has high type I error rates in the presence of time or behavioral variation. The Stanley and Burnham (1999) test allows for time variation, but rejects at greater than nominal error rates when heterogeneity or behavioral variation is present. Neither test is accurate with the presence of behavioral variation because trap shy behavior is indistinguishable from emigration; the same is true for trap happy behavior and immigration (Stanley and Burnham 1999).

As the program CAPTURE uses a closed-population model, an important assumption is that for the duration of the experiment the population is closed to birth, death, immigration, and emigration. Based on the short duration of the study, I assumed the population was closed. While general closure was assumed, traps on the perimeter of the study site most likely captured animals with home ranges that only partially overlapped the study area and could therefore inflate the population density estimate. To account for this and promote closure, an estimate of the effective trapping area (larger than the trapping grid) should be used to provide a more reliable density estimate (Otis et al. 1982). I calculated the effective trapping area by measuring 1/2 of the mean

maximum distance traveled by all bears visiting >1 trap and adding that distance to the perimeter of the study area (Otis et al. 1978).

I compared the annual density estimates after the population estimates and density calculations were completed for both 2006 and 2007. The consistency of estimates in the genetic tagging study was determined by comparing the 95% confidence intervals (CI) of each estimate for overlap. I also compared the genetic tagging density estimates to the regional population estimates determined by the NHFG using harvest and mortality data. To do this, I examined whether the NHFG density estimates fell within the 95% CI of the genetic tagging estimates. If so, I concluded that the estimation methods produced similar results.

## Descriptive Population Statistics

As population estimates for each study area were taken in 2 consecutive years, I hypothesized that the allele frequencies and genetic patterns would be similar in both years. Therefore, to avoid redundancy in the data sets, I used only the data from 2006 for all descriptive statistics, structural, and spatial genetic analyses.

### Hardy-Weinberg Equilibrium

Gene or allele frequencies are the fundamental parameter of population genetics. The allele frequency indicates the proportion of alleles of a gene that are identical in the population (Hartl and Clark 2007). Allele frequencies are calculated to determine if a population is in genetic equilibrium, which is an important assumption in population genetics and is described by the Hardy-Weinberg principle. The Hardy-Weinberg principle states that the allele frequencies of a sexually reproducing population will remain stable if the population 1) is large, 2) has random mating, 3) has negligible

mutation and migration, and 4) is not subject to selection (Hartl and Clark 2007). Deviations from equilibrium occur when there is inbreeding, assortative mating (mates that have more similar or dissimilar traits than predicted by chance), the existence of null alleles, and natural selection (Hartl and Clark 2007).

As Hardy-Weinberg equilibrium is an important assumption in most population genetics analyses, I tested for equilibrium in each study site using the program FSTAT (version 2.9.3.2; Goudet 1995). This approach tests for the random union of gametes to determine if the allele frequencies are in agreement with the Hardy-Weinberg expectations. FSTAT uses a randomization test with 1000 permutations to test for Hardy-Weinberg equilibrium and the Bonferroni adjustment to determine statistical significance in the presence of multiple tests.

**Linkage Disequilibrium**

When a population is in Hardy-Weinburg equilibrium, the alleles at each locus are randomly associated with one another. If not, alleles may be "linked" in a process called linkage disequilibrium (Hartl and Clark 2007). While the microsatellite loci I used in this study have been shown to be independent of one another (Paetkau and Strobek 1994, Paetkau et al. 1995) this equilibrium needs to be demonstrated for each population studied. The detection of linkage could indicate sampling bias, sampling of siblings, the presence of immigrants, or the occurrence of stochastic processes occurring in my study (Thompson 2003). I tested for linkage disequilibrium using the program FSTAT that uses the log-likelihood ratio G-test with 600 permutations and Bonferroni adjustment for multiple tests to determine if the alleles at 1 locus are independent from alleles at another locus.

## Relatedness

Identifying the relationship (or relatedness) between individuals is useful for describing social organization and detecting population structure. Relatedness can be used on a fine scale to establish genealogies, or on a larger scale to help determine the extent of spatial genetic structure in a population. Relatedness can be described as the probability that genotypes of 2 individuals share 0, 1, or 2 alleles that recently descended from an ancestral allele (identity by descent; Blouin 2003). In biologically relevant terms, relatedness values are a continuous measure that fall between 0-1; for example, non-related individuals exhibit a relatedness value of 0 (zero probability they share an allele), siblings have a relatedness value of 0.5 (50% probability that they share an allele), and an individual has a relatedness of 1 with itself (100% probability that it shares the same allele). Each possible relationship between individuals (e.g., parent-offspring, grandparent-grandchild) has a predicted relatedness value, though several relationships have the same relatedness value and are difficult to distinguish.

There are several different estimators to determine relatedness, and traditionally these were based on method-of-moments statistics (Queller and Goodnight 1989, Lynch and Ritland 1999). Drawbacks to these traditional estimators are that the relatedness values are not constrained to fit within the biologically relevant range (0-1), and the estimators are undefined for 2 equally frequent allele frequencies (Milligan 2003). A newer approach to estimating relatedness is to use the method of maximum likelihood estimation. In general, these methods estimate the parameter value that maximizes the probability of obtaining the observed data for a given model (Allendorf and Luikart 2007). For assessing relatedness, this translates to estimating the relationship (and/or

relatedness value) with the maximum likelihood, given the observed genotypes. Relatedness estimators have large variances, and while Blouin (2003) recommends 30-40 microsatellite loci to obtain moderate confidence, other studies have used 7-14 loci for adequate power in brown and black bears (Onorato et al. 2004, Cronin et al. 2005, Moyer et al. 2006). While relatedness values calculated with few loci aren't especially useful for identifying genealogies, they can be valuable in estimating the proportion of each type of relationship category that occurs in a sample and for testing hypotheses about which populations are more closely related on average.

ML-RELATE (Kalinowsky et al. 2006) is a computer program that uses maximum likelihood to estimate pairwise relatedness values and identify the most probable relationships between individuals. I used the program ML-RELATE to identify the pairwise relatedness values for individuals within the populations to describe the proportion of relationship categories. I then calculated and compared the average relatedness values for subpopulations using a relatedness matrix from the program ML-RELATE in the PopMeans function of the program GENALEX (Peakall and Smouse 2006). This function essentially provides an estimate of average relatedness for each subpopulation relative to the population as a whole. I used this function to compare the average relatedness of males and females. Because females are the philopatric sex, females should be significantly more related to each other than to males or the population on average. Females are also expected to have higher average relatedness than males. To test this prediction on my data set, I used a Wilcoxin signed rank test in the program JMP (SAS Institute) to test for difference in the average relatedness values of males and

females within each study site, and I pooled the study sites for a broader analysis of male and female relatedness.

## Population Structure and Spatial Genetic Patterns

### Population Structure

Populations can be thought of as hierarchical and are generally divided into smaller units or subpopulations. While subpopulations are not usually genetically isolated from one another, they may display differentiation in allele frequencies caused by genetic drift (Hartl and Clark 2007). This genetic differentiation between subpopulations is referred to as population structure. Allele frequencies can therefore be used in conjunction with F statistics to determine if subpopulations are differentiated. F statistics were developed by Wright (1921) and are a measure of the deficit of heterozygotes relative to the expected Hardy-Weinberg proportions in a population (Allendorf and Luikart 2007). Heterozygote deficits are expected when a population ceases to mate randomly, for example, as a result of population substructure. F statistics, therefore, describe the amount of inbreeding or non-random mating in a population. Of particular importance to population studies is the statistic $F_{ST}$ that is a measure of divergence in allele frequencies between subpopulations. This statistic helps determine whether 2 putative subpopulations are connected by gene flow, and subsequently are functioning as 1 larger mating population, or are genetically distinct. To determine whether bears in Pittsburg and Milan were connected by gene flow, I used the program FSTAT to calculate the $F_{ST}$ (Weir and Cockerham 1984) between the Pittsburg and Milan populations.

In population genetics several different methods are often used concurrently to test hypotheses, because different methods have different assumptions that can lead to different results (Bergl and Vigilant 2007, Rowe and Beebee 2007). When using more than one method, results are stronger if there is a consensus. Therefore, in addition to calculating $F_{ST}$, I used several other methods for identifying population differentiation. I used the program STRUCTURE version 2.1 (Pritchard et al. 2000) that employs a Bayesian clustering approach to estimate the number of subpopulations (K) within a data set, without defining populations *a priori*. This approach assigns individuals to populations based on their individual multilocus genotypes (Dawson and Belkhir 2001, Manel et al. 2003, Coulon et al. 2006). By assigning individuals to distinct populations, biologists can define management units and also identify landscape features that inhibit gene flow (Manel et al. 2003, Guillot et al. 2005). Pritchard et al. (2000) first used Bayesian clustering to determine distinct populations of the Taita flush (*Turdus helleri*), an endangered African bird species. Since then, similar techniques have been used with the Moroccan argan tree (*Argania spinosa*; Corander et al. 2003) and European roe deer (*Capreolus capreolus*; Coulon et al. 2006). I used STRUCTURE to infer the number of potential subpopulations (K) for the combined Pittsburg-Milan data set by conducting 5 independent runs for K = 1-5, using a burn-in period of 500,000 replications, and $10^6$ Markov chain Monte Carlo steps assuming a model of admixture. I also used the PopMeans relatedness function to compare the average relatedness of the Pittsburg and Milan populations. If Pittsburg and Milan are separate populations, each should be significantly more related to itself than to the combined population on average.

## Spatial Genetic Structure

Population structure as described above identifies genetic units, while spatial genetic structure explores the association of individuals in space through their genetic relatedness. This spatial genetic structure can correlate with behavior and social organization, or landscape features that limit gene flow. To characterize spatial genetic structure, I tested for the presence of isolation by distance and spatial autocorrelation. Isolation by distance studies seek to determine whether there is a significant relationship between genetic and geographic distance (Wright 1943). I used a Mantel test in the program GENALEX to determine if isolation by distance was present in the study population. A Mantel test is a simple correlation method that determines the presence of a statistical relationship between 2 distance matrices, in this case a genetic and geographic distance. A positive correlation indicates that as the geographic distance between individuals increases, so does the genetic distance, demonstrating that as individuals are spaced further apart, they are less related. A negative correlation indicates that as geographic distance increases, genetic distance decreases (relatedness increases).

Spatial autocorrelation is a fine scale and more powerful investigation of isolation by distance that tests the significance of the correlation (geographic distance and relatedness) at specific distance classes (Peakall et al. 2003). The autocorrelogram shows the distance class to which significant positive correlation occurs. In effect, individuals found within distances smaller than the significant positive correlation share a higher proportion of genes, and individuals more distant than this threshold are genetically independent. Spatial autocorrelation is typically used to examine fine-scale genetic

patterns and from these infer the biological processes such as dispersal that generate these patterns (Double et al. 2005). I first tested for spatial autocorrelation in each study site separately, then pooled the study sites for an increased sample size, and finally analyzed the spatial autocorrelation in males and females separately.

**Landscape Resistance to Gene Flow**

To determine if landscape features such as roads and rivers affected gene flow, I used the PopMeans function in the program GENALEX to compare average relatedness of subpopulations separated by Route 3 in Pittsburg and the Route 16-Androscoggin River corridor in Milan. As Route 3 and the Route 16-Androscoggin River corridor run North-South, in both areas I labeled the subpopulations East and West. If these landscape features influence gene flow, the subpopulation on either side of the barrier should be more related to itself than when compared to the whole. To see if males and females respond to these landscape features differently, I analyzed each sex independently. For males caught on both sides of the landscape features, I included their genotypes in the analysis of both subpopulations.

# CHAPTER 3

# RESULTS

## Discrimination of Individuals

### Probability of Identity

Based on the recommendations from the 2003 study, 6 highly variable loci were used in the genetic analysis to ensure that no 2 individuals had the same genotype. The $P_I$ and $P_{Isib}$ values across the 6 loci were low in both study sites for both years ($P_I$: $1.1E^{-8}$-$3.73E^{-8}$; $P_{Isib}$: $1.7E^{-3}$-$2.2E^{-3}$; Table 1) suggesting strong individual detection. In addition, all individual multilocus genotypes met the $P_{sib}$ "match" test rejection criterion of $P_{sib}<0.05$ and were included in the population estimate.

### Genotyping Error

I re-analyzed 94 hair samples (8% of total) to estimate the rate of genotyping error. Two samples were discarded because they failed to amplify. In total, 1288 alleles were compared and 41 genotyping errors occurred for an error rate of 3% per allele. Four errors were due to allelic dropouts and 37 due to scoring error. This scoring error was usually a shift of 1 repeat length, when an allele is scored as a single repeat away from the reference genotype. The error rate varied across loci from 0-24% with a mean of 5% (Table 2). Most of the error derived from 2 loci (G10B, G10L) and may be attributed to consistent mis-scoring at one allele in G10B, and a large number of alleles leading to greater possibility of mis-scoring in G10L. The error rate per multilocus genotype was 37% in absence of the error-checking protocol.

**Table 1.** The probability of identity ($P_I$) and probability of sibling ($P_{Isib}$) statistics. Significantly(*) low p-values are indicative of powerful loci: $P_I$ ($P<0.005$), and $P_{Isib}$ ($P<0.01$).

| Study Area and Year | $P_I$ | $P_{Isib}$ |
|---|---|---|
| Pittsburg | | |
| 2006 | 1.1E-8* | 1.7E-3* |
| 2007 | 2.7E-8* | 2.0E-3* |
| Milan | | |
| 2006 | 1.8E-8* | 1.9E-3* |
| 2007 | 3.73E-8* | 2.2E-3* |

**Table 2.** Error rates per locus, per allele, and per multilocus genotype as estimated from a re-analysis of 94 (8%) hair samples from 2007. These error rates were calculated without precautionary screening. Other black bear genetic tagging studies have shown an error rate per locus of 0-4% (Paetkau 2003).

| Locus | Error rate/locus (%) |
|---|---|
| G1A | 0 |
| G10B | 12 |
| G10C | 0 |
| G1D | 2 |
| G10L | 24 |
| G10X | 2 |
| SRY (41F & 121R; Taberlet et al. 1993) | 0 |
| SRY (Bellemain and Taberlet 2004) | 1 |
| **Average** (± SE) | 5.1 ± 3.0 |
| Error rate/allele | 3.1 |
| Error rate/multilocus genotype | 37.0 |

## Population Estimation

### Pittsburg: Year One

I collected 1,790 hair samples during the field season (5 June-27 July 2006). The mean trap success rate (% of traps visited by bears per session) was 56%. Trap activity peaked by session 5 and the number of samples collected weekly ranged from 138-305. This corresponds to an average of 4.5 hair samples per trap, per week (Table 3). Sub-sampling resulted in the analysis of 395 DNA samples. Forty-one (10%) samples were discarded due to lack of DNA amplification, and 13 (3%) were discarded because they contained hair from more than 1 individual. The remaining 341 samples were comprised of 67 unique genotypes (individuals). In constructing a capture history for the 67 individuals, redundancy in captures within the same trap and same trap session were collapsed to represent a single capture per trapping session (Table 4). The 67 individuals were thus captured a total of 170 times. Of the 67 individuals, 37 (53%) were captured more than once (up to 8 captures of 1 individual). New individuals (4-16) were captured each trap session with the highest rates (8-16) occurring in the initial 4 sessions (Table 3). The male to female sex ratio was 34M:33F; the sex of 2 individuals were not identifiable.

The program CAPTURE was used to select the appropriate model to estimate population size. Initially, data from 8 trap sessions were used to estimate the population. The model selection procedure detected varying capture probabilities by individual animal (heterogeneity model, $M_h$; $X^2 = 2.255$, $df = 3$, $P<0.00001$) and a behavioral response to capture (behavioral model, $M_b$; $X^2 = 13.071$, $df = 1$, $P = 0.0003$). I did not detect a variation of capture probability by time or trap session (time variation model, $M_t$;

**Table 3.** Summary statistics describing black bear hair trapping in Pittsburg (N = 50 trap sites) New Hampshire, summers of 2006 and 2007.

| Year<br>Trap Session | Traps with hair samples | Hair samples (#) | Hair samples per trap (Mean ± SE) | New bears captured |
|---|---|---|---|---|
| 2006 | | | | |
| 1 | 15 (30%) | 156 | 3.1 ± 0.67 | 16 |
| 2 | 19 (38%) | 201 | 4.0 ± 0.87 | 10 |
| 3 | 27 (54%) | 258 | 5.2 ± 0.89 | 8 |
| 4 | 32 (64%) | 267 | 5.3 ± 0.81 | 11 |
| 5 | 37 (74%) | 273 | 5.5 ± 0.75 | 5 |
| 6 | 34 (68%) | 305 | 6.1 ± 0.88 | 7 |
| 7 | 30 (60%) | 192 | 3.8 ± 0.87 | 4 |
| 8 | 30 (60%) | 138 | 2.8 ± 0.52 | 6 |
| Total | 224 | 1790 | | 67 |
| Mean ± SE | 28 ± 2.6 (56%) | 223.8 ± 21.3 | 4.5 ± 0.78 | 8 ± 1.4 |
| 2007 | | | | |
| 1 | 28 (56%) | 209 | 4.2 ± 1.10 | 24 |
| 2 | 29 (58%) | 161 | 3.2 ± 0.52 | 10 |
| 3 | 34 (68%) | 255 | 5.1 ± 0.81 | 12 |
| 4 | 37 (74%) | 112 | 2.2 ± 0.28 | 4 |
| 5 | 42 (84%) | 307 | 6.1 ± 0.82 | 8 |
| 6 | 39 (78%) | 217 | 4.3 ± 0.59 | 7 |
| 7 | 33 (66%) | 155 | 3.1 ± 0.54 | * |
| 8 | 26 (52%) | 127 | 2.5 ± 0.51 | * |
| Total | 268 | 1543 | | 65 |
| Mean ± SE | 34 ± 2.0 (68%) | 192.9 ± 23.5 | 3.9 ± 0.47 | 11 ± 2.9 |

*Samples only genotyped for 6 trap sessions

**Table 4.** Summary of the number of hair samples, individual bears, sex ratio and recapture rate per number of trap sessions.

| Study Area Year | Trap Sessions | Samples Analyzed | Individual Bears | Sex Ratio (M:F) | Recapture Rate (%) |
|---|---|---|---|---|---|
| Pittsburg | | | | | |
| 2006 | 8 | 341 | 67 | 34:33 | 54 |
| | **5** | **213** | **50** | **24:24** | **60** |
| 2007 | 6 | 268 | 65 | 34:31 | 65 |
| | **5** | **217** | **58** | **31:27** | **62** |
| Milan | | | | | |
| 2006 | 8 | 284 | 81 | 51:27 | 44 |
| | **5** | **198** | **56** | **35:20** | **34** |
| 2007 | 6 | 218 | 68 | 44:24 | 51 |
| | **5** | **178** | **59** | **39:20** | **47** |

$X^2 = 6.454$, df = 7, P = 0.48782). The goodness of fit tests indicated a good fit for the heterogeneity model $M_h$ ($X^2 = 7.787$, df = 7, P = 0.35173) and a poor fit for the behavioral model $M_b$ ($X^2 = 20.620$, df = 12, P = 0.05624). Because the goodness of fit for behavioral response was poor, I did not consider a strictly behavioral model (see Appendix A for estimates from all models).

Conversely, because individual heterogeneity was detected and had a good fit, I considered jackknife ($M_h$-jackknife) and Chao ($M_h$-Chao) models. Models $M_h$-jackknife and $M_h$-Chao produced similar population estimates of 107 (SE = 13.9283, CI = 88-144, CV = 13%) and 104 bears (SE = 17.8418, CI = 83-157, CV = 17%). While the $M_h$-Chao model is useful for estimating the size of a population with low capture probability, fewer recaptures, and a low sample size, it lacks precision and accuracy and has a wide confidence interval. I therefore selected the $M_h$-jackknife model (Table 5) as the best estimator because it is known to be robust in the presence of other sources of variation (behavior/time) and because the study exhibited a number of recaptures and a reasonable probability of capture (p = 0.1986; Chao 1989, Mowat & Strobeck 2000, Miller et al. 2005).

In 2006, the mean maximum distance traveled by bears visiting >1 trap was approximately 4.8 km (3.0 mi). Therefore, a buffer area of approximately 2.4 km (1.5 mi) was added to the edge of each peripheral trap, resulting in an effective trapping area of 387.5 $km^2$ (149.6 $mi^2$). The estimated population density in the effective trapping area based on the $M_h$-jackknife estimate was 0.28 bears/$km^2$ (0.72 bears/$mi^2$). The range of the density estimate based on the 95% confidence interval was 0.21-0.35 bears/$km^2$ (0.53-0.90 bears/$mi^2$; Table 5).

**Table 5.** Summary of population estimates of black bears in northern New Hampshire, 2006 and 2007.

| Study Area | No. Trap Sessions | Model | Estimate | SE | Estimate 95% CI | Mean Capture Probability | Density (bears/km$^2$) | Density 95% CI |
|---|---|---|---|---|---|---|---|---|
| Pittsburg | | | | | | | | |
| 2006 (388 km$^2$) | 8 | Mh-Jackknife | 107 | I3.9 | 88-144 | 0.20 | 0.28 | 0.21-0.35 |
| **2006 (315 km$^2$)** | **5** | **Mbh-Pollock** | **70** | **10.0** | **58-100** | **0.21** | **0.22** | **0.16-0.28** |
| 2007 (407 km$^2$) | 6 | Mh-Jackknife | 83 | 7.1 | 74-102 | 0.30 | 0.20 | 0.17-0.24 |
| **2007 (400 km$^2$)** | **5** | **Mh-Jackknife** | **78** | **8.3** | **67-101** | **0.33** | **0.20** | **0.15-0.24** |
| Milan | | | | | | | | |
| 2006 (440 km$^2$) | 8 | Mbh-Pollock | 123 | 18.3 | 100-176 | 0.05 | 0.28 | 0.20-0.36 |
| **2006 (440 km$^2$)** | **5** | **Mth-Chao** | **106** | **25.0** | **76-182** | **0.15** | **0.24** | **0.13-0.35** |
| 2007 (398 km$^2$) | 6 | Mbh-Pollock | 113 | 16.4 | 97-129 | 0.10 | 0.28 | 0.20-0.36 |
| **2007 (371 km$^2$)** | **5** | **Mbh-Pollock** | **99** | **14.1** | **80-137** | **0.10** | **0.27** | **0.19-0.34** |

Ideally in mark-recapture studies the number of newly captured individuals is expected to decrease with time, but the data did not fit this pattern. New individuals were captured at a high rate in all 8 weeks, suggesting closure violation. Possible explanations for this include immigration into the study sites, movement of transient males through the study sites, and changes in seasonal bear movements in response to food availability causing bears to be caught at traps outside their core home range. These factors could result in the capture of additional bears in later trapping sessions that were not present in the initial weeks of the study. If these late captures are nonresident bears that are traveling through the study site, then an estimate based on 8 trap sessions will be inflated with respect to the resident population. To this end, I evaluated my dataset to determine if it would be more appropriate to use less than 8 trapping sessions to generate the population estimate.

First, I considered the question of geographic closure. While CAPTURE assumes the population is closed, the study area may be open to limited immigration or emigration. I tested for closure with the 8 week data sets using the program CLOSETEST; because there was heterogeneity, the Otis et al. (1978) test was used. Results indicated that the Pittsburg study area was in violation of closure ($z = -2.62$, $P = 0.004$). Because closure violation might be minimized by short sampling periods (White et al. 1982, Lancia et al. 1994, Greenwood and Robinson 2006), I reviewed the pattern of new captures for a pattern of decline in new individuals captured, and truncated the capture history to encourage demographic closure. By removing the later capture sessions, immigrants and transients are eliminated from the data set, thereby facilitating a more appropriate fit to the mark-recapture models (White et al. 1982). The study period

of 5 trap sessions was chosen for subsequent population estimation because the number of new captures decreased until the fifth trap session and then fluctuated in trap sessions 6-8 (Table 3). Using data from only the first 5 trap sessions eliminates new bears caught in later trapping sessions and therefore provides a conservative population estimate of the likely resident individuals. Data from the 5 trap sessions yielded 50 individuals and a sex ratio of 24M:24F.

The program CAPTURE was then used to analyze the data from the first 5 trap sessions (Appendix B). The model selection procedure detected heterogeneity ($X^2$ = 7.64, df = 2, P = 0.022), and "trap happy" behavioral response ($X^2$ = 7.54, df = 1, P = 0.006), and did not detect temporal variation in capture probability ($X^2$ = 9.14, df = 5, P = 0.10). The goodness of fit tests found both the heterogeneity model and the behavioral model to be a good fit. The $M_{bh}$ model only uses the probability of first capture to estimate population size because subsequent recaptures are influenced by a behavioral response. To eliminate the effect of the "trap happy" or "trap shy" bias, only the initial capture probability is used to produce an estimate and the data are treated as if each individual were captured once and then removed from the population (White et al. 1982). There are 2 estimators for the $M_{bh}$ model: the "generalized removal estimator" ($M_{bh}$-Removal; Otis et al. 1978) and $M_{bh}$-Pollock (Pollock and Otto 1983). These 2 estimators are known to perform differently. The $M_{bh}$-Removal estimator typically has a negative bias when there is high heterogeneity in capture probability and has a larger standard error with fewer sampling sessions. As $M_{bh}$-Pollock is known to be more precise with fewer sampling sessions, it is favored for practical use (Pollock and Otto 1983). The

$M_{bh}$-Pollack model was selected as the best estimator because there was an indication of both heterogeneity and behavior influencing capture probability.

The $M_{bh}$-Pollack model predicted 70 bears (SE = 10, CI = 58-100, CV = 14%) and may be a better estimate for the number of resident bears rather than the larger estimate (107 ± 14) based on the data from 8 trap sessions. The mean maximum distance traveled by bears visiting >1 trap was 3.8 km (2.4 mi) that translates to an effective trapping area of 315.3 $km^2$ (122.7 $mi^2$). Based on the effective trap area, the population density was estimated to be 0.22 bears/$km^2$ (0.57 bears/$mi^2$), with a range of 0.16-0.28 bears/$km^2$ (0.41-0.73 bears/$mi^2$) based on the 95% confidence interval (Table 5). As expected, the population estimate and density declined when the study period was reduced to 5 trap sessions. To test for closure, I executed CLOSETEST while bearing in mind that CAPTURE detected both heterogeneity and behavioral variation. This means that the more appropriate closure test is Otis et al. (1978), and due to the presence of behavioral variation, the test has high type I error rates (false detection of closure violation). Results of the closure test for 5 trap sessions indicated that the study area was still not closed (z = -2.02, P = 0.02).

**Pittsburg: Year Two**

I collected a total of 1,543 hair samples at 50 trap sites in 8 weekly sessions during summer 2007 (4 June-26 July, 2007). Because the year 1 analyses indicated that fewer trap sessions were more appropriate to ensure closure, only a sub-sample from the first 6 trap sessions was genotyped. Of the 287 genotyped samples, six (2%) were discarded due to lack of DNA amplification, and 13 (5%) were discarded because they contained hair from more than 1 individual. Sixty-five unique individuals were captured

a total of 152 times (Table 4). Of the 65 individuals, 42 (65%) were captured more than once (up to 8 captures of 1 individual). New individuals (4-24) were captured each trap session with the highest rates occurring in the first 3 sessions (Table 3). The sex ratio of males to females was approximately even, 34M:31F.

Initially, I used the data from 6 trap sessions in the mark-recapture analysis (Appendix C). Heterogeneity ($X^2 = 9.92$, df = 2, P = 0.007) and behavioral variation ($X^2 = 5.421$, df = 1, P = 0.02) were detected, but time variation was not ($X^2 = 7.903$, df = 5, P = 0.16). The goodness of fit test indicated that the heterogeneity model was the best fit ($X^2 = 13.675$, df = 10, P = 0.19); therefore, the $M_h$-jackknife estimator was used. The $M_h$-jackknife model predicted 83 bears (SE = 7.1, CI = 74-102, CV = 9%; Table 5). I tested for closure using the Otis et al. (1978) test that functions in the presence of heterogeneity and found that the study area was not closed for the 6-week period (z = -2.99, P = 0.001). The average maximum distance moved by bears was 5.1 km (3.2 mi), resulting in an effective trapping area of 406.6 $km^2$ (157.0 $mi^2$) that was slightly larger than in 2006. The population density was estimated to be 0.20 bears/$km^2$ (0.53 bears/$mi^2$), with a range of 0.17-0.24 bears/$km^2$ (0.44-0.62 bears/$mi^2$), based on the 95% confidence interval (Table 5).

To ensure demographic closure and aim for consistency between yearly comparisons, I reviewed the capture history for 5 trap sessions and tested it for closure (Otis et al. 1978). I found that the study area was closed for the 5-week period (z = -1.17, P = 0.12). The capture history over 5 weeks fit the assumptions of closure better than the 6-week data and was therefore used in subsequent population estimation (Appendix D). The capture history for 5 trapping sessions identified 58 individuals and a sex ratio of

31M:27F (Table 4). The model selection procedure in CAPTURE detected heterogeneity of capture probability ($X^2 = 6.3$, df = 2, P = 0.04) and a behavioral response ($X^2 = 3.0$, df = 1, P = 0.08). The goodness of fit tests indicated a good fit for the heterogeneity and behavioral models. I selected the $M_h$-jackknife model as the appropriate model and it produced an estimate of 78 bears (SE = 8.3, CI = 67-101, CV = 11%). The effective trapping area was 399.9 $km^2$ (154.4 $mi^2$) and the revised estimated population density was 0.20 bears/$km^2$ (0.51 bears/$mi^2$) [range: 0.15-0.24 bears/$km^2$ (0.40-0.61 bears/$mi^2$)], similar (9% lower) to that of the previous year (0.22 bears/$km^2$; Table 5).

**Milan: Year One**

I collected a total of 1,350 hair samples at 51 trap sites in 8 weekly sessions during summer 2006 (29 May-20 July, 2006). A subset of samples was analyzed resulting in 328 genotyped samples. Thirty-four (10%) samples were discarded due to lack of DNA amplification, and 10 (3%) were discarded because they contained hair from more than 1 individual. Eighty-one unique individuals were captured a total of 149 times (Table 6). Of the 81 individuals, 36 (44%) were captured more than once (up to 8 captures of 1 individual). New individuals (6-17) were captured each trap session with the highest rates (11-17) occurring in the middle 4 sessions (Table 6). The sex ratio of males to females was 51M:27F; the sex of 3 individuals was not identifiable.

I initially used the data from 8 trap sessions in the mark-recapture analysis (Appendix E). The model selection procedure in CAPTURE detected heterogeneity of capture probability ($X^2 = 7.93$, df = 2, P = 0.019), temporal variation in capture probability ($X^2 = 27.19$, df = 7, P = 0.0003), and a behavioral response ($X^2 = 11.76$, df = 1, P = 0.001). In addition, recaptures were significantly greater than the first capture

**Table 6.** Summary statistics describing black bear hair trapping in Milan (N = 51 trap sites) New Hampshire, summers of 2006 and 2007.

| Year<br>Trap Session | Traps with hair samples | Hair samples (#) | Hair samples per trap (Mean ± SE) | New bears captured |
|---|---|---|---|---|
| 2006 | | | | |
| 1 | 20 (39%) | 104 | 1.0 ± 0.53 | 8 |
| 2 | 18 (35%) | 125 | 2.5 ± 0.69 | 9 |
| 3 | 24 (47%) | 159 | 3.1 ± 0.61 | 13 |
| 4 | 34 (67%) | 279 | 5.5 ± 0.91 | 17 |
| 5 | 23 (45%) | 183 | 3.6 ± 0.66 | 9 |
| 6 | 27 (53%) | 143 | 2.8 ± 0.58 | 11 |
| 7 | 32 (63%) | 210 | 4.1 ± 0.71 | 8 |
| 8 | 25 (49%) | 147 | 2.9 ± 0.56 | 6 |
| Total | 203 | 1350 | | 81 |
| Mean ± SE | 25 ± 1.9 (49%) | 168.8 ± 19.5 | 3.3 ± 0.66 | 10 ± 1.2 |
| 2007 | | | | |
| 1 | 26 (51%) | 109 | 2.1 ± 0.49 | 15 |
| 2 | 31 (61%) | 131 | 2.6 ± 0.50 | 12 |
| 3 | 29 (57%) | 129 | 2.5 ± 0.40 | 10 |
| 4 | 39 (76%) | 115 | 2.3 ± 0.28 | 12 |
| 5 | 40 (78%) | 205 | 4.0 ± 0.55 | 10 |
| 6 | 38 (75%) | 123 | 2.4 ± 0.33 | 9 |
| 7 | 21 (41%) | 63 | 1.2 ± 0.27 | * |
| 8 | 28 (55%) | 52 | 1.0 ± 0.16 | * |
| Total | 252 | 927 | | 68 |
| Mean ± SE | 32 ± 2.4 (63%) | 115.8 ± 16.5 | 2.3 ± 0.32 | 11 ± 0.9 |

*Samples only genotyped for 6 trap sessions

probabilities indicating "trap happy" behavior, in which bears return to a hair trap after their first encounter. The goodness of fit tests indicated a good fit for the behavior model. While all 3 sources of variation were detected, there is no model with a corresponding estimator for $M_{bht}$. Given that the goodness of fit for behavioral response was strong, I considered models that incorporated heterogeneity ($M_{bh}$) and time ($M_{tb}$) in addition to behavioral variation.

Estimators $M_{bh}$-Pollock and $M_{tb}$-Burnham both produced a population estimate of 123 bears ($M_{bh}$-Pollock: SE = 18, CI = 100-176, CV = 15%; $M_{tb}$-Burnham: SE = 52, CI = 88-361, CV = 43%). I selected the $M_{bh}$-Pollock estimate as it had a lower standard error. I did not consider the $M_{bh}$-removal estimator because of its known biases and lack of precision; it produced an inflated population estimate with a large SE (Appendix E). To identify if immigration and/or emigration occurred during the 8 weeks, I used the program CLOSETEST to check for closure violation (Otis et al 1978). This test indicated that the study area was not closed ($z = -2.21$, $P = 0.01$), but it may be biased due to the presence of behavioral variation in capture probability. The mean maximum distance traveled by bears in Milan was approximately 6.4 km (4 mi) that produced an effective trapping area of 439.8 $km^2$ (169.8 $mi^2$) and an estimated population density of 0.28 bears/$km^2$ (0.72 bears/$mi^2$), [range: 0.20-0.36 bears/$km^2$ (0.51-0.94 bears/$mi^2$); Table 5].

In an effort to uphold the assumption of closure and to encourage consistency of the estimates, I calculated an estimate based on 5 trap sessions (Appendix F); 56 individuals were identified and the sex ratio was still unbalanced with 35M:20F. Temporal variation in capture probability was detected ($X^2 = 19.25$, $df = 4$, $P = 0.001$).

The model selection procedure failed to detect heterogeneity, and had insufficient data to test a behavioral response when compared to the null model. While the data were insufficient, it is likely that a behavioral response was still present as it was detected in the presence of heterogeneity ($X^2 = 13.09$, df = 6, P = 0.042). The goodness of fit test indicated that the behavioral model was better than the heterogeneity model; insufficient data existed to test the fit of the time variation model. The $M_{th}$-Chao model was selected as the appropriate estimator and produced an estimate of 106 bears (SE = 25, CI = 76-182, CV = 24%; Table 5).

I used the program CLOSETEST to determine if the reduction of trapping sessions promoted closure, but because the capture probability showed variation due to time and heterogeneity, the appropriate closure test was difficult to determine. The Otis et al. (1978) closure test works in the presence of heterogeneity but not for time, while the Stanley and Burnham (1999) test works for time and not heterogeneity. I therefore used both tests to test for closure and found inconsistent results. According to the Otis et al. (1978) test, the study area was not closed (z = -2.74, P = 0.003), while the Stanley and Burnham test (1999) showed that the study area was closed ($X^2 = 9.14$, df = 5, P = 0.10). The effective trapping area was 439.8 $km^2$ (169.8 $mi^2$) and the estimated population density based on the data from 5 trap sessions was 0.24 bears/$km^2$ (0.62 bears/$mi^2$), [range: 0.13-0.35 bears/$km^2$ (0.34-0.91 bears/$mi^2$); Table 5].

**Milan: Year Two**

I collected a total of 927 hair samples at 51 trap sites in 8 weekly sessions during summer 2007 (28 May-19 July, 2007; Table 6). While hair samples were collected for 8 trap sessions, my previous analyses indicated that fewer trap sessions might be more

appropriate to ensure closure, therefore genetic analysis was only conducted on samples from the first 6 trap sessions. A subset of samples was analyzed from the first 6 trap sessions resulting in 227 genotyped samples. Five (2%) samples were discarded due to lack of DNA amplification, and 4 (2%) were discarded because they contained hair from more than 1 individual. Sixty-eight unique individuals were captured a total of 131 times. Of the 68 individuals, 35 (51%) were captured more than once (up to 8 captures of 1 individual). New individuals (9-15) were captured each trap session (Table 6). The sex ratio of males to females was male biased: 44M:24F.

Population estimation was first conducted on data from 6 trap sessions (Appendix G). The model selection procedure in CAPTURE detected the presence of heterogeneity ($X^2 = 12.564$, df = 2, P = 0.002) and behavioral variation ($X^2 = 10.705$, df = 1, P = 0.001), but not temporal variation ($X^2 = 7.795$, df = 5, P = 0.17) in capture probability. Recapture probability was greater than the first capture probabilities, indicating "trap happy" behavior. The goodness of fit tests indicated that the $M_{bh}$ model was the best fit. I therefore used the $M_{bh}$-Pollock estimator that predicted 113 bears (SE = 16.4, CI = 91-158, CV = 15%). I tested for closure using the test from Otis et al. (1978) and found the study area was not closed (z = -2.05, P = 0.02). The mean maximum distance moved by bears was 5.3 km (3.3 mi), producing an effective trapping area of 397.8 $km^2$ (153.6 $mi^2$). The estimated population density of bears was 0.28 bears/$km^2$ (0.74 bears/$mi^2$), [range: 0.20-0.36 bear/$km^2$ (0.53-0.94 bears/$mi^2$); Table 5].

I reduced the number of trapping sessions to 5 to encourage closure and consistency (Appendix H). Fifty-nine individuals were identified in 5 trap sessions, and the revised sex ratio was male biased at 39M:20F. The model selection procedure in

CAPTURE detected heterogeneity of capture probability ($X^2$ = 14.798, df = 1, P<0.001), and a "trap happy" behavioral response ($X^2$ = 8.529, df = 1, P = 0.004). The goodness of fit tests indicated a good fit for the heterogeneity and behavioral models. I selected the $M_{bh}$-Pollock (Pollock and Otto1983) model as the appropriate model and it produced an estimate of 99 bears (SE = 14.1, CI = 80-137, CV = 14%; Table 5). The Otis et al. (1978) closure test was performed and found the study area was not closed (z = -1.6, P = 0.05). The effective trapping area was 371.2 $km^2$ (143.3 $mi^2$) and the estimated population density was 0.27 bears/$km^2$ (0.69 bears/$mi^2$), similar to that conducted the previous year (0.24 bears/$km^2$), [range: 0.19-0.34 bears/$km^2$ (0.50-0.88 bears/$mi^2$); Table 5].

## Descriptive Population Statistics

### Hardy-Weinberg Equilibrium and Linkage Disequilibrium

To test for Hardy-Weinberg equilibrium, allele frequencies were calculated for all individuals in Pittsburg and Milan. At each locus, 8-12 alleles were observed in Pittsburg and 7-14 alleles were observed in Milan. Allele frequencies ranged from 0.007-0.31 in Pittsburg and 0.006-0.43 in Milan (Table 7). No deviations from Hardy-Weinberg were detected in either population before or after the Bonferroni correction (adjusted P value<0.008). No loci were linked in either population before or after the Bonferroni correction (adjusted P value<0.001).

### Relatedness

The Pittsburg population was composed of paired individuals (dyads) that were 81% unrelated, 16% half-sibling, 2% full sibling, and 2% parent-offspring. This was similar in Milan with 81% unrelated, 15% half-sibling, 2% full sibling, and 2% parent-offspring dyads. When considering males and females separately in both Pittsburg and

**Table 7.** Observed allele frequencies of black bears identified from hair samples taken in 2006 in Pittsburg and Milan, New Hampshire.

| Locus (Allele length) | Pittsburg (N = 67) | Milan (N = 81) |
|---|---|---|
| G1A | | |
| 171 | 0.007 | 0.062 |
| 173 | 0.22 | 0 |
| 175 | 0 | 0.012 |
| 183 | 0 | 0.179 |
| 185 | 0.06 | 0.08 |
| 187 | 0.134 | 0.173 |
| 189 | 0.246 | 0.216 |
| 191 | 0.291 | 0.179 |
| 193 | 0.134 | 0.093 |
| 195 | 0.097 | 0.006 |
| 197 | 0.007 | 0 |
| G10B | | |
| 153 | 0.015 | 0 |
| 155 | 0.052 | 0.062 |
| 157 | 0.075 | 0.259 |
| 159 | 0.284 | 0.074 |
| 161 | 0.067 | 0.21 |
| 163 | 0.157 | 0.13 |
| 165 | 0.119 | 0.154 |
| 167 | 0.149 | 0.111 |
| 169 | 0.082 | 0 |
| G10C | | |
| 96 | 0 | 0.031 |
| 98 | 0.119 | 0.019 |
| 100 | 0.067 | 0.086 |
| 102 | 0.067 | 0.062 |
| 104 | 0.06 | 0.031 |
| 106 | 0.06 | 0.012 |
| 108 | 0.246 | 0.296 |
| 110 | 0.246 | 0.309 |
| 112 | 0.09 | 0.117 |
| 116 | 0.007 | 0.037 |
| 118 | 0.037 | 0 |

**Table 7.** (Continued)

| Locus (Allele length) | Pittsburg (N = 67) | Milan (N = 81) |
|---|---|---|
| G1D | | |
| 170 | 0.007 | 0.031 |
| 172 | 0.06 | 0.012 |
| 174 | 0.313 | 0.426 |
| 176 | 0.179 | 0.222 |
| 178 | 0.127 | 0.056 |
| 180 | 0.194 | 0.123 |
| 182 | 0.052 | 0.093 |
| 184 | 0.067 | 0.037 |
| G10L | | |
| 128 | 0.007 | 0.006 |
| 134 | 0 | 0.148 |
| 136 | 0.172 | 0.093 |
| 138 | 0.119 | 0.037 |
| 140 | 0.142 | 0.019 |
| 144 | 0 | 0.037 |
| 150 | 0.007 | 0.056 |
| 152 | 0 | 0.111 |
| 154 | 0.157 | 0.179 |
| 156 | 0.142 | 0.105 |
| 158 | 0.104 | 0.08 |
| 160 | 0.06 | 0.111 |
| 162 | 0.067 | 0.012 |
| 164 | 0.015 | 0 |
| 166 | 0 | 0.006 |
| 170 | 0.007 | 0 |
| G10X | | |
| 177 | 0.007 | 0.006 |
| 179 | 0 | 0.006 |
| 183 | 0.075 | 0.031 |
| 185 | 0.187 | 0.228 |
| 187 | 0.306 | 0.241 |
| 189 | 0.06 | 0.012 |
| 191 | 0.037 | 0.019 |
| 193 | 0.097 | 0.136 |
| 195 | 0.112 | 0.16 |
| 197 | 0.015 | 0.019 |
| 199 | 0.007 | 0.031 |
| 201 | 0.007 | 0.019 |
| 203 | 0.09 | 0.08 |
| 205 | 0 | 0.012 |

Milan, the percent of dyads in each category was similar between the sexes (Table 8).

Considering the average relatedness of males and females in Pittsburg, the PopMeans function in GENALEX established that relatedness in males and females was not greater within each sex as compared to the whole population (males: $r = 0.08$, $P = 0.20$; females: $r = 0.07$, $P = 0.76$; Fig. 7). Similarly in Milan, the PopMeans function in GENALEX indicates males and females do not differ in mean relatedness (males: $r = 0.08$, $P = 0.23$; females: $r = 0.07$, $P = 0.56$; Fig. 8). I also used a Wilcoxin-signed rank test to test for a difference in the mean relatedness of males and females. This test pooled relatedness values from both study sites and showed no difference between the 2 means ($z = -0.98$, $P = 0.33$), indicating that the average relatedness of males and females was similar.

## Population Structure and Spatial Genetic Patterns

### Population Structure

Using F statistics, I detected population structure between Pittsburg and Milan. The $F_{ST}$ value was 0.024 ($P = 0.05$), indicative of a small but significant genetic difference between the 2 populations. Comparison of individual relatedness values in the PopMeans function of GENALEX also indicated that the 2 study sites were genetically distinct. Individual bears in both Pittsburg ($r = 0.09$, $P<0.0001$) and Milan ($r = 0.09$, $P<0.001$) were significantly more related within each subpopulation than to the population as a whole, suggesting genetic differentiation of these populations (Fig. 9). The program STRUCTURE, however, did not detect population structure between Pittsburg and Milan. In the 5 independent simulations of the Bayesian clustering method, the most probable number of genetic clusters was $K = 1$, with an average logarithm

**Table 8**. An analysis of relatedness using maximum-likelihood methods for bears in 2006 in Pittsburg and Milan, New Hampshire. Reported are the number and percent of paired individuals (dyads) in the relatedness categories of: unrelated (U), half siblings (HS), full siblings (FS), and parent-offspring (PO).

| | Total | Males | Females |
|---|---|---|---|
| ***Pittsburg*** | | | |
| No. dyads | 2211 | 528 | 496 |
| % U | 81 | 80 | 81 |
| % HS | 16 | 17 | 15 |
| % FS | 2 | 2 | 2 |
| % PO | 2 | 1 | 2 |
| ***Milan*** | | | |
| No. dyads | 3240 | 1275 | 351 |
| % U | 81 | 81 | 82 |
| % HS | 15 | 15 | 14 |
| % FS | 2 | 2 | 2 |
| % PO | 2 | 2 | 2 |

**Figure 7.** Mean relatedness of female (N = 33) and male (N = 34) bear subpopulations in Pittsburg as compared to the population as a whole using the PopMeans function in GENALEX. Gray bars are the 95% confidence interval bounding the null hypothesis of "no difference" between populations, and error bars about the mean are determined through bootstrapping. Significant within group relatedness can be assumed when mean r exceeds the 95% CI.

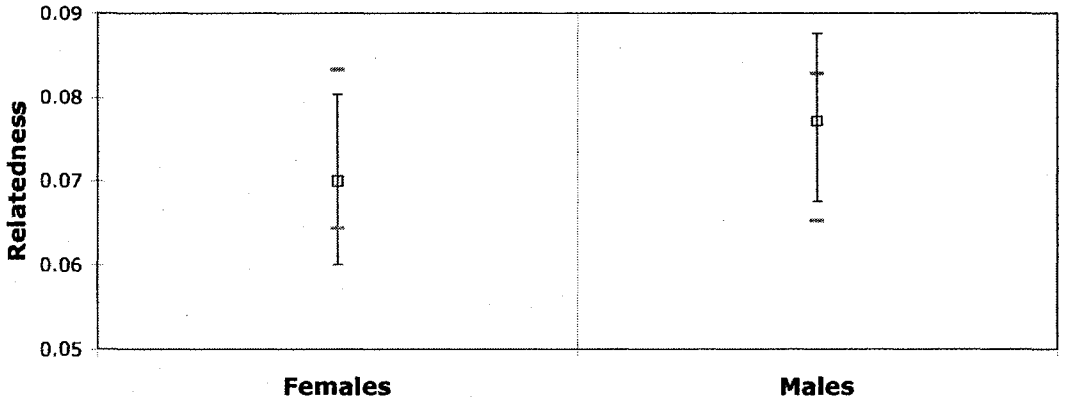


**Figure 8.** Mean relatedness of female (N = 27) and male (N = 51) bear subpopulations in Milan as compared to the population as a whole using the PopMeans function in GENALEX. Gray bars are the 95% confidence interval bounding the null hypothesis of "no difference" between populations, and error bars about the mean are determined through bootstrapping. Significant within group relatedness can be assumed when mean r exceeds the 95% CI.

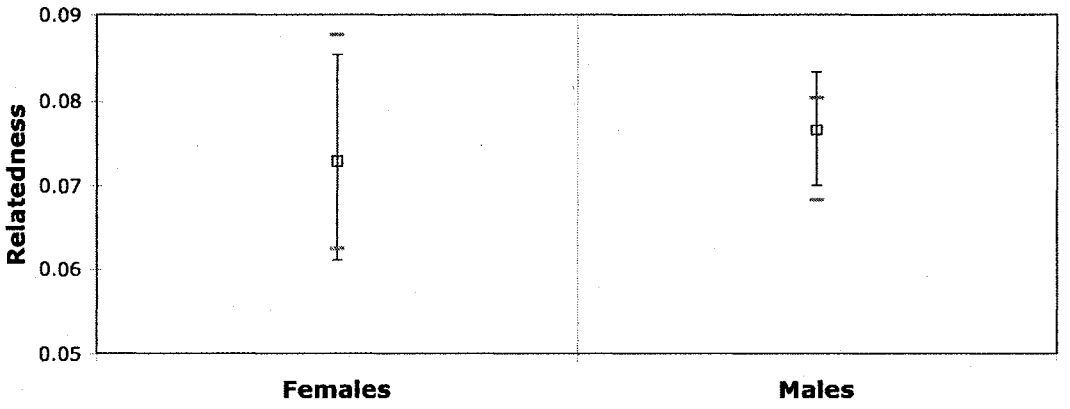
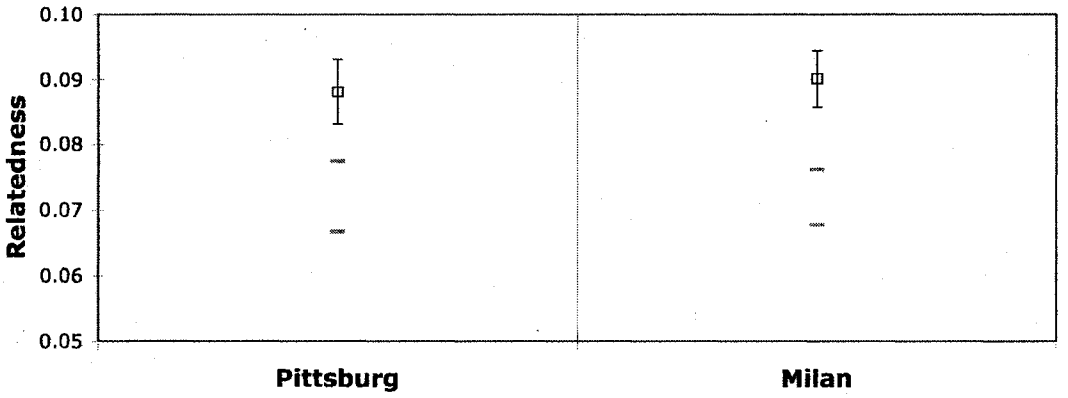


**Figure 9.** Mean relatedness of Pittsburg (N = 67) and Milan (N = 81) subpopulations as compared to the population as a whole using the PopMeans function in GENALEX. Gray bars are the 95% confidence interval bounding the null hypothesis of "no difference" between populations, and error bars about the mean are determined through bootstrapping. Significant within group relatedness can be assumed when mean r exceeds the 95% CI.



probability of the data ln Pr(X|K) = -3666 (Fig. 10). Not only did K = 1 maximize probability of the data, but variation increased as K increased.

**Spatial Genetic Structure**

A Mantel test indicated a positive correlation between the geographic and genetic distance (R = 0.13, N = 148, P<0.0001) across the combined populations, indicating that as distance between individuals increased, their relatedness decreased (Fig. 11). Spatial autocorrelation analysis found no significant spatial structure within Pittsburg or Milan when tested alone, but when pooled to increase sample size, significant positive spatial structure was found in the 2 and 4 kilometer distance classes (Fig. 12). The x-intercept for *r* was 8.8 km (5.4 mi), indicating a positive genetic correlation among individuals within this distance.

I also conducted a separate spatial autocorrelation analysis for males and females pooled across the 2 study sites. As expected, males showed no positive spatial structure regardless of the distance class (Fig. 13). Females exhibited positive spatial structure in the 2 and 4 km distances classes, with an x-intercept of 5.9 km (3.7 mi; Fig. 13).

**Landscape Resistance to Gene Flow**

In the male subpopulations separated by Route 3 in Pittsburg, the East (r = 0.07, P = 0.21) and the West (r = 0.04, P = 0.78) had similar relatedness values and were not genetically distinct (Fig. 14). In females, the West had higher relatedness (r = 0.09, P = 0.13) than the East (r = 0.06, P = 0.59), but there was no genetic distinction between the 2 subpopulations (Fig. 15). This suggests that for both sexes, the subpopulations East and West of Route 3 are connected by gene flow. In Milan, East (r = 0.08, P = 0.41) and West (r = 0.09, P = 0.06) males had similar relatedness and were not genetically distinct

**Figure 10.** The results of the STRUCTURE analysis. The estimated number of populations is taken to be the value of K (number of populations) at which the probability is maximized. The plot shows the likelihood of each value of ln Pr(X|K) from 5 independent runs for K = 1-5. The probability was maximized for K = 1.

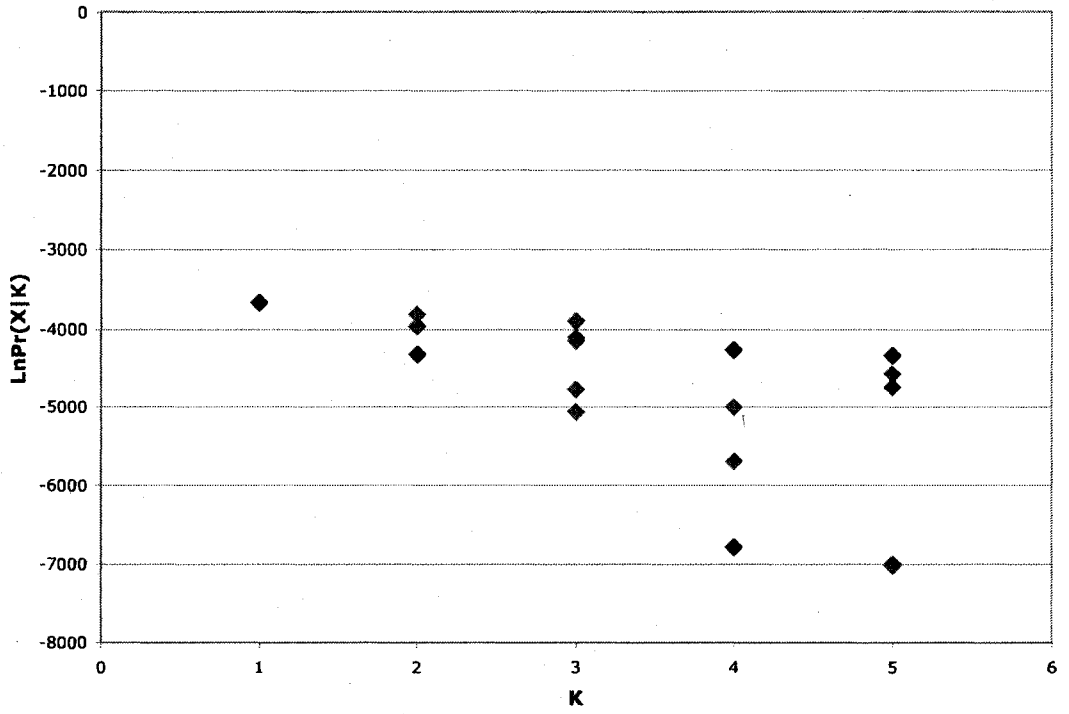


**Figure 11.** A Mantel test illustrating the positive relationship between geographic distance (km) and genetic distance in the combined study sites of Pittsburg and Milan, New Hampshire (R = 0.13, N = 148, P<0.0001).

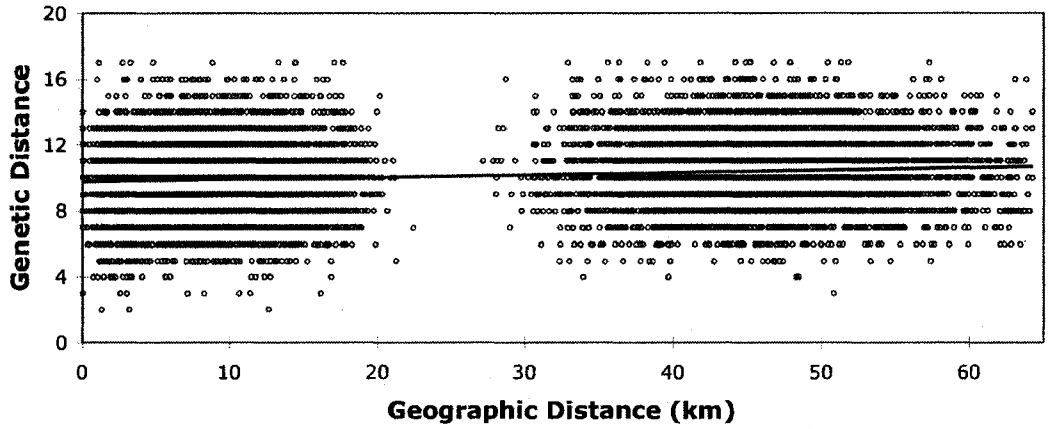


**Figure 12.** Correlogram plot of the genetic correlation coefficient (*r*) as a function of distance for the pooled populations of Pittsburg and Milan, New Hampshire (N = 148). The null hypothesis of no spatial autocorrelation is bounded by the permuted 95% confidence interval (dashed lines) that is determined from permuting individual genotypes across geographic distance classes. Error bars for mean *r* at each distance class were estimated with bootstrapping. Significant spatial autocorrelation can be assumed when mean *r* exceeds the 95% CI. The x-intercept for *r* was 8.8 km and this corresponds to the distance beyond which there is no genetic correlation.

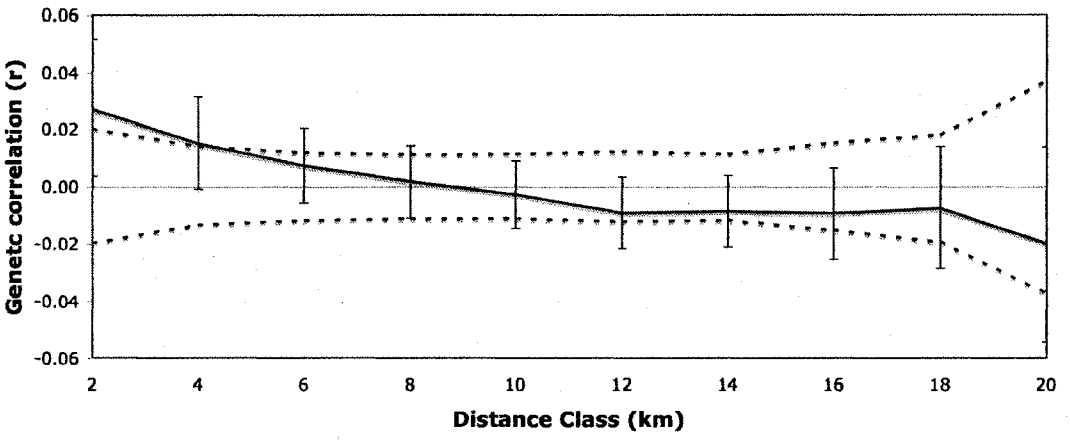


**Figure 13.** Correlogram plot of the genetic correlation coefficient ($r$) as a function of distance in the populations of Pittsburg and Milan, New Hampshire. Males (N = 85) and females (N = 60) were pooled for the 2 populations and analyzed separately. The null hypothesis of no spatial autocorrelation is bounded by the permuted 95% confidence interval (dashed lines) that is determined from permuting individual genotypes across geographic distance classes. Error bars for mean $r$ at each distance class were estimated with bootstrapping. Significant spatial autocorrelation can be assumed when mean $r$ exceeds the 95% CI. The x-intercept for $r$ was 5.9 km in females and 9.9 km in males, and this corresponds to the distance beyond which there is no genetic correlation.

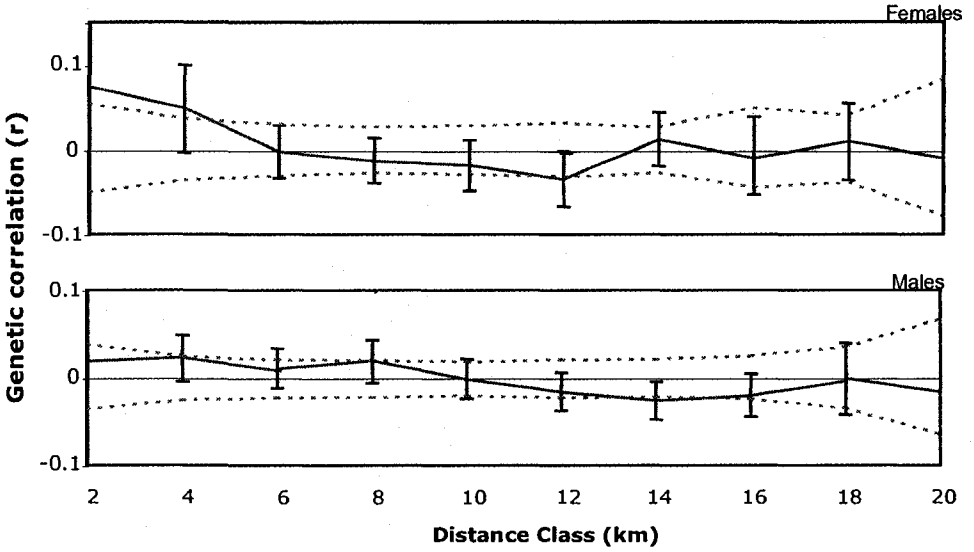


**Figure 14.** Mean relatedness of male bear subpopulations East (N = 29) and West (N = 5) of Route 3 in Pittsburg, NH as compared to the population as a whole using the PopMeans function in GENALEX. Gray bars are the 95% confidence interval bounding the null hypothesis of "no difference" between populations, and error bars about the mean are determined through bootstrapping. Significant within group relatedness can be assumed when mean r exceeds the 95% CI.

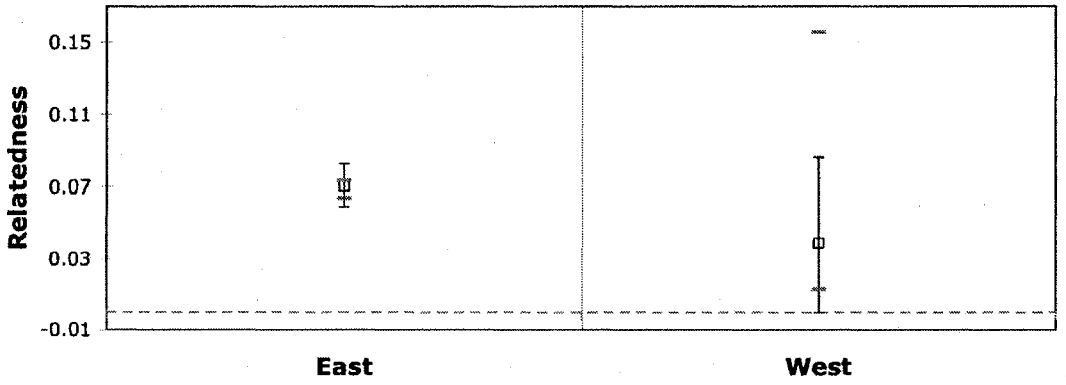
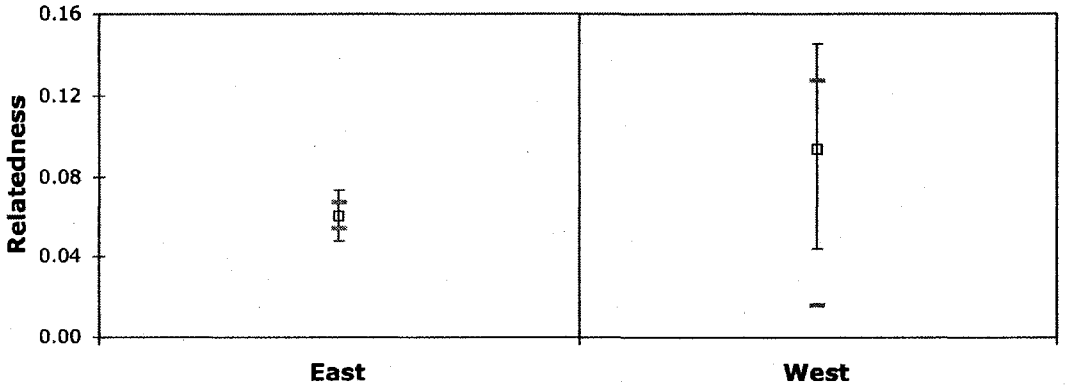


**Figure 15.** Mean relatedness of female bear subpopulations East (N = 26) and West (N = 6) of Route 3 in Pittsburg, NH as compared to the population as a whole using the PopMeans function in GENALEX. Gray bars are the 95% confidence interval bounding the null hypothesis of "no difference" between populations, and error bars about the mean are determined through bootstrapping. Significant within group relatedness can be assumed when mean r exceeds the 95% CI.



(Fig. 16). In contrast, females in the East were genetically distinct from the larger population ($r = 0.07$, $P = 0.05$), while those in the West were not ($r = 0.04$, $P = 0.91$; Fig. 17).

**Figure 16.** Mean relatedness of male bear subpopulations East (N = 30) and West (N = 25) of the Route 16-Androscoggin River corridor in Milan, NH as compared to the population as a whole using the PopMeans function in GENALEX. Gray bars are the 95% confidence interval bounding the null hypothesis of "no difference" between populations, and error bars about the mean are determined through bootstrapping. Significant within group relatedness can be assumed when mean r exceeds the 95% CI.

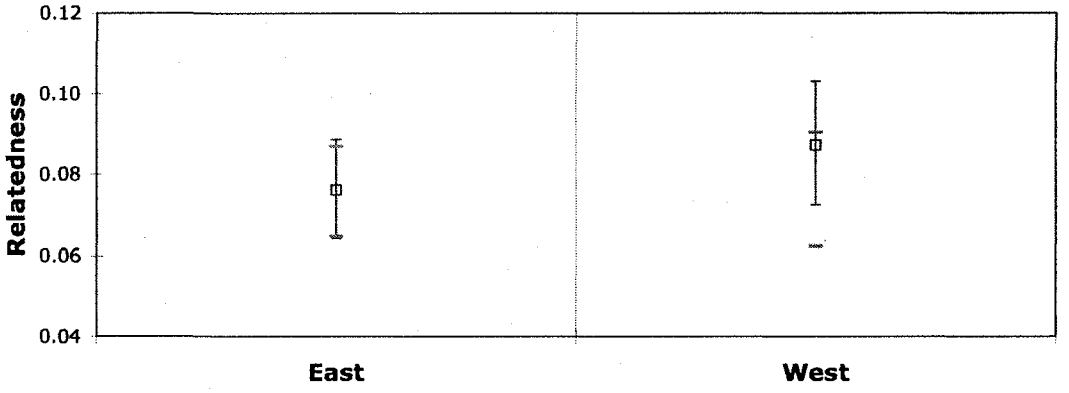
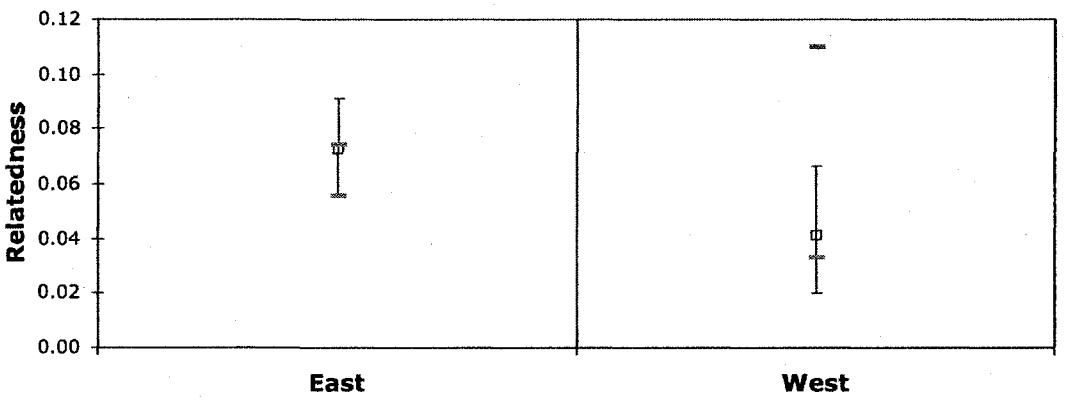


**Figure 17.** Mean relatedness of female bear subpopulations East (N = 20) and West (N = 8) of the Route 16-Androscoggin River corridor in Milan, NH as compared to the population as a whole using the PopMeans function in GENALEX. Gray bars are the 95% confidence interval bounding the null hypothesis of "no difference" between populations, and error bars about the mean are determined through bootstrapping. Significant within group relatedness can be assumed when mean r exceeds the 95% CI.



# CHAPTER 4

# DISCUSSION

## Discrimination of Individuals

The probability to detect individuals using 6 loci was high, indicating strong individual identification. The mean per locus genotyping error rate was 5%, and the error rate per multilocus genotype was 37%. These error rates are consistent with other studies of black bears. Paetkau (2003) reported an average error rate per locus of 0-3% in 18 bear studies while Dreher et al. (2007) reported an error rate per locus of 4% and an error rate per multilocus genotype of 20%. A low percentage of genotyping errors often leads to a high percentage of multilocus genotypes with at least 1 error (Bonin et al. 2004). Importantly, these rates represent the error without any filtering, repetition, or screening for potential inaccuracies. Although error rates of 5% per locus can bias CAPTURE estimates by >200% (Waits and Leberg 2000, Roon et al. 2005) when single and double mismatches were error checked, bias in CAPTURE estimates is reduced to <5% (Roon et al. 2005). I am confident that by reanalyzing all homozygote and suspect genotypes, having a single person analyze samples, and manually reviewing all similar genotypes, sufficient precautions were taken to minimize genotyping error. After using the aforementioned error checking protocol, there were no 2 samples that displayed a mismatch at 1 locus, further supporting that this protocol succeeded at both detection and correction of genotyping errors.

## Population Estimation

### Closure Violation

An important assumption of the program CAPTURE is that for the duration of the experiment the population is closed to birth, death, immigration, and emigration. After testing for closure using the program CLOSETEST, I found a consistent lack of closure that prompted me to shorten the sampling period to encourage closure. While black bear birth and death can be virtually excluded from the duration of the 2-month study period, changes in bear movement may result in immigration and emigration from the study area. These movements may include the dispersal of yearlings after family break up, transient males crossing the study site, or changes in individual movement patterns in response to seasonal forage. If these movements are systematic throughout the season, then their timing should be considered in relation to the timing of a mark-recapture study. Mark-recapture studies should therefore be designed with consideration of these seasonal movements, as they can impact the estimated density.

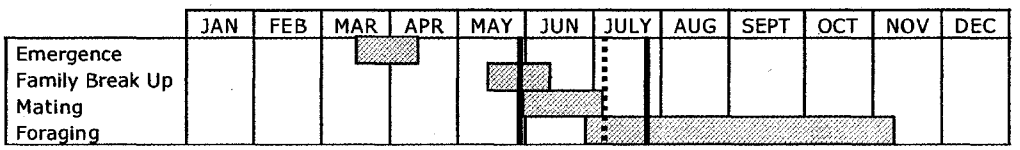
Typically, bear movement throughout the year can be described in stages: emergence, mating, foraging, and denning (Rogers 1987). Home range of both males and females is well defined during the mating period (Garshelis and Pelton 1981, Rogers 1987, Inglis and Wilton 1998). After mating bears enter into the foraging period that occurs from early July-November. During this period, wide ranging travel outside their home range is common for both males and females as bears are attracted to rich feeding sites that correspond to high fruit or nut availability (Garshelis and Pelton 1981, Rogers 1987). An ideal time to conduct a mark-recapture study coincides with the mating period because it facilitates conservative estimation of the abundance of bears in the mating

population of that area. To reduce the risk of an inflated estimate due to movement, mark-recapture studies should be conducted during the mating period and prior to the foraging period.

In New Hampshire family break up occurs in late May-early June and overlaps with the beginning of the mating season (Fig. 18). The mating season runs from late May-early July (Ben Kilham, NH bear rehabilitator, pers. comm. 2008) when soft mast begins to appear and the foraging period starts. This study began in early June and ran through mid-late July, therefore, the 8-week trapping session probably extended beyond the mating season and encompassed movement associated with the foraging period. The 5-week trapping session, however, was in early June-early July and corresponded to the mating season. Based on the bear biology in New Hampshire, therefore, the timing of the 5-week session was more appropriate in meeting the assumptions of the mark-recapture models used in this study.

After truncating the trap sessions to 5 weeks, and re-running CAPTURE, the program CLOSETEST found lack of closure in Pittsburg in 2006 and closure in 2007. In Milan the populations were never closed. In both study sites, however, the models selected in CAPTURE had behavioral and temporal variation and CLOSETEST does not perform well for either source of variation (Stanley and Burnham 1999). Realistically, it is likely complete closure will never occur and so I believe the estimates based on 5 week trapping sessions more accurately reflect the number of bears in the mating population and therefore the resident population, because they represent conservative estimates taken during a sampling period with presumably less movement and subsequently less closure violation. In addition, truncating the trapping sessions increased the capture probabilities

**Figure 18.** Stages and timing of bear movement in New Hampshire based on personal communication with Ben Kilham, NH black bear rehabilitator (2008). Solid vertical lines represent the 8-week period in which hair trapping took place in this study for both 2006 and 2007, while the dotted line represents the 5-week trapping session.



(Table 5). The 5-week trapping period was also used in population estimate for the 2007 trapping season to encourage consistency between the years.

In the presence of closure violation, one might argue that an open population model may be more appropriate. However, the open Jolly-Seber model is unbiased only if all movement corresponds to permanent transient movement (i.e., 1 entry and 1 exit; Kendall 1999). As temporary movement is more likely in a short study such as this one, the closed population model is more appropriate (Boulanger et al. 2002). Also, the open Jolly-Seber model is not robust to the presence of heterogeneity in capture probabilities (Gilbert 1973), yet heterogeneity was an important response in these populations. All genetic tagging studies of bears to date have been conducted using closed population models (Mowat and Strobeck 2000, Poole et al. 2001, Boersen et al. 2003, Thompson 2003, Belant et al. 2005, Dreher et al. 2006, Immel and Anthony 2006, Settlage et al. 2006).

The size of the trapping grid in this study was reduced from 13 to 5 square kilometer (5 to 2 square miles) cells because of the male biased sex ratio in the 2003 pilot study. The increased number of traps per female home range was successful in increasing the proportion of captured females. However, Boulanger et al. (2002) noted that there is a tradeoff when using smaller grid cells in genetic tagging studies. They suggested that smaller trapping grids result in more precise estimates due to increased capture probabilities, but at the risk of closure violation (Boulanger et al. 2002, Boulanger et al. 2004). If bear movement is temporary, the net result of closure violation is that the population estimate corresponds to a superpopulation of bears in the grid and

the surrounding area (Kendall 1999). To adjust for this, I added a perimeter to the study site equal to 1/2 the mean maximum distance traveled by all bears visiting >1 trap.

In an ideal mark-recapture study, the number of newly captured individuals should decrease and recaptures should increase over time. I saw no such trend as new individuals in both study sites were captured in the later sessions and contributed to the detection of closure violation. If the late captures were due primarily to changes in movement of peripheral or adjacent bears, then the new individuals should generally be caught in traps on the edge of the trapping grid. A review of the capture history of both years indicated that most new individuals captured late in the trapping season (sessions 5-8) were caught on the edge of the trapping grid. The edge was defined as all traps on the perimeter of the study site, and it should be noted that due to the study design more than 50% of the traps are edge traps. The near-edge traps were all traps within 3.2 km (2 mi) of an edge trap, and a center trap was defined as those traps >3.2 km (2 mi) from the edge. In Pittsburg 70% of the new individuals captured in trapping sessions 6-8 were caught in traps on the edge of the grid, 27% were caught in traps near the edge, and 3% were caught in central traps. Similarly, in Milan 66% of the new individuals captured in the last trapping sessions were caught on the edge, 19% were caught near the edge, and 15% were caught in the center. This illustrates that most new captures late in the trapping season were either residents on the periphery of the trapping grid, or immigrants coming from outside the trapping grid. This edge response was predicted and partially corrected for by calculating a larger effective trapping area.

I predicted that the majority of the new individuals caught late in the trapping season would be males because of their larger movements (Rogers 1987). In Pittsburg,

this was not true, as the new captures were evenly split between the sexes (49% male and 51% female). In Milan, males were a larger percentage of new captures (64% male and 30% female, 6% undetermined). It is interesting to note that these ratios closely match the overall sex ratio at each study site. This congruence suggests that new bears caught late in the trapping season had home ranges that overlapped the trapping grid and reflected the local population. They are likely captured later in the trapping season as they increase their movements outside of their core home range in response to seasonal food availability. This type of movement should affect both sexes similarly, consistent to these findings.

**Program CAPTURE Models**

While the population and density estimates were consistent in the consecutive sampling seasons, the models and estimators selected by the program were not. In Pittsburg the $M_{bh}$ model and Pollock estimator were selected in 2006 and the $M_h$ model and jackknife estimator were selected in 2007. This indicates that in Pittsburg in 2006 variation in the capture probabilities was affected by the presence of both behavior and heterogeneity and in 2007 variation was affected by heterogeneity alone. In Milan, the $M_{th}$ model and Chao estimator was selected in 2006 and the $M_{bh}$ model and Pollock estimator was selected in 2007. Therefore, variation in capture probabilities were affected by time and heterogeneity in 2006 and they were affected by behavior and heterogeneity in 2007. As the mean capture probability ranged from 0.10-0.33, this study did not capture every bear in the study area. It is therefore expected that the sampled individuals were different in each sampling year and would express different capture probabilities, leading to different models and estimators chosen for population estimation.

The models account for the variation in the capture probabilities and adjust for this in the estimation (White et al. 1982); therefore the difference in model selection in the 2 years should not affect the population estimates.

A trap happy behavioral response was a common source of variation detected in the capture probabilities. This was not surprising given that the traps were baited with a food reward of flaked corn. However, this modification from the 2003 study (when no bait was used) was successful in increasing the number of hair samples collected. In 2003, a total of 126 hair samples were collected in the 6-week study period, while an average of 1300 samples were collected at each study site in 2006 and 2007. This 10-fold sample increase translated into greater selectivity of samples for optimum DNA yield, higher capture probabilities, and a larger percentage of the true population being sampled. Ideal capture probabilities for the mark-recapture algorithms are at least 10%, and preferably 20% (White et al. 1982). The modifications to the study design increased capture probabilities from 0.07 in 2003 to more favorable capture probabilities of 0.10-0.33 in this study (Table 5). Also, this behavioral response did not affect the population estimate as the program CAPTURE was able to detect this behavioral response and select a model and estimator that accounted for this variation.

**Population Density Estimates**

Both the genetic tagging population estimates and the estimates derived from hunter-harvest methods used by the NHFG are indirect measurements of a true population parameter (N). As characteristic of an estimate, the relationship to the true parameter tends to vary (Greenwood and Robinson 2006). The Paloheimo and Fraser (1981) estimate based on hunter harvest data, mortality data, and bear observation rates is

influenced by the number and enthusiasm of hunters, as well as the detectability of bears due to weather conditions, yearly food resource quality, and individual bear behavior. Similarly, the genetic tagging estimate is influenced by factors including bear behavior, resource quality, and heterogeneity. Because the estimates were derived from different methods that each respond to differences in bear detectability, they are difficult to compare. However, a comparison was made by examining whether the NHFG density estimates were within the 95% CI of the genetic tagging estimates.

The density of bears in Pittsburg in 2006 was 0.22 bears/km$^2$ (0.57 bears/mi$^2$) and 0.20 bears/km$^2$ (0.51 bears/mi$^2$) in 2007. In 2006 in Milan the density of bears was 0.24 bears/km$^2$ (0.62 bears/mi$^2$) and 0.27 bears/km$^2$ (0.69 bears/mi$^2$) in 2007. The bear densities estimated in 2 consecutive years at each study site were nearly identical, with overlapping 95% confidence intervals indicating no detectable population change in the consecutive years (Table 5).

The NHFG density estimates are derived from mortality data to calculate harvest rates and hunter observation rates are then used to estimate regional populations. Pittsburg and Milan are both in the north region and the density estimates were 0.22-0.25 bears/km$^2$ in 2005-2007 (NHFG Federal Aid Reports 2006, 2007, 2008; Table 9). The genetic tagging population estimates in Pittsburg were about 9% lower (0.20-0.22 bears/km$^2$) than the NHFG estimates, whereas the Milan estimates were about 13% higher (0.24-0.27 bears/km$^2$, Table 9). The NHFG density estimate (0.23 bears/km$^2$) fell within the 95% confidence interval of the genetic tagging estimate in Pittsburg in 2006, but not 2007 (0.25 bears/km$^2$). In Milan the NHFG density estimate fell within the 95%

**Table 9.** Density estimates from the New Hampshire Fish and Game Department based on traditional mortality statistics for the north region of New Hampshire (NHFG Federal Aid Reports 2006, 2007, 2008) compared to density estimates for Pittsburg and Milan, NH generated from the genetic tagging study.

| Year | Density estimate (bears/km$^2$) | CI* |
|---|---|---|
| 2005 | | |
| NHFG | 0.22 | 0.20-0.25 |
| 2006 | | |
| NHFG | 0.23 | 0.21-0.26 |
| Pittsburg | 0.22 | 0.16-0.28 |
| Milan | 0.24 | 0.13-0.35 |
| 2007 | | |
| NHFG | 0.25 | 0.23-0.27 |
| Pittsburg | 0.20 | 0.15-0.24 |
| Milan | 0.27 | 0.19-0.34 |

*The confidence interval for the NHFG density estimate is 80%, while for the genetic tagging study it is 95%

confidence interval in both years (2006: 0.13-0.35 bears/km$^2$; 2007: 0.19-0.34 bears/km$^2$; Table 9).

The density estimate in Pittsburg from the 2003 pilot study (0.16 bears/km$^2$; 0.41 bears/mi$^2$; 95% CI 0.10-0.21 bears/km$^2$) was approximately 24% lower than the densities estimated in this study (Kovach and Pekins 2004). The density estimates for Pittsburg in 2006 does not fall within the 95% CI from 2003, while the 2007 estimate does. The capture probability was lower at 0.07 and the standard error of the estimate was greater (SE of 17 compared to a mean SE of 9 in this study), indicating less confidence in the 2003 density estimate. Presumably, the methodological improvements made in the study led to a more precise estimate.

The differences in the densities of the Pittsburg and Milan populations (>20%) may be related to relative food availability. Nutrition in the form of hard and soft mast is an important factor in reproductive success (Elowe and Dodge 1989). American beech (*Fagus grandifolia*) and northern red oak (*Quercus rubra*) are the 2 most important hard mast producing species for black bears in New Hampshire (Timmins 2004). Although beech is present in Pittsburg, oak is absent due to climatic conditions (Pease 1964, Frieswyk and Widmann 2000). In Milan, however, loamy soils in the Mahoosuc Mountain Range support both beech and red oak (Polak et al. 2007). The presence of red oak in Milan presumably offers additional food resources for black bears. As the annual yield of hard mast is variable, summer soft mast may be a more important determinant of habitat quality, and increased clear-cutting in Milan encourages the growth of soft mast in early successional habitats (A. Timmins, NH Bear Project Leader, pers. comm. 2008).

These food resources translate into better quality habitat and may contribute to higher bear densities in Milan.

When compared to other black bear population estimates in the US, the estimates from Pittsburg and Milan fall in the middle of the range. With a similar sized study area (329 $km^2$) in Louisiana, Boerson et al. (2003) estimated bear density to be 0.36 bears/$km^2$. Also, Immel and Anthony (2008) found a mean density of 0.21 bears/$km^2$ in their 2 Oregon study sites. It is important to note, however, that bear density is dependent on resource quality (Rogers 1987). In studies that undertook to estimate a population density on a more regional scale, bear densities were much smaller. Dreher et al. (2007) estimated bear densities for an area of 36,848 $km^2$ in Michigan to be 0.05 bears/$km^2$, Settlage et al. (2008) found a mean bear density of 0.02 ± 0.01 bears/$km^2$ for a mean area of 11,173 ± 2,780 $km^2$ in the southeastern US. Higher black bear densities have also been recorded on islands in the Apostle Islands National Lakeshore in Wisconsin where mean bear density was 0.57 ± 0.07 bears/$km^2$ (Belant et al. 2005).

On a regional scale, the traditional density estimates may be more cost effective as there is minimal overhead cost. In addition, the similarity in densities between the 2 methods indicates that the traditional methods are probably sufficient to provide a regional or WMU density estimate. However, the genetic tagging methods improve the regional approach by detecting demographic differences between local populations. Therefore if there are management concerns at a local scale due to increased hunter access or other demographic changes, the genetic tagging method provides a more precise density estimate. Further, this method detects imbalance in local sex ratios as identified in Milan.

On a regional scale however, genetic tagging studies of black bears note monetary and logistical challenges to establishing and checking sufficiently high number of traps for precise population estimates (Settlage et al. 2008). Modifications to the genetic tagging protocol have been suggested to alleviate such logistical challenges and facilitate the application of these techniques on a regional scale (Dreher et al. 2007). Modifications include the elimination of a grid design and instead sampling based on historic levels of harvest, incorporating tissue from harvest bears as a recapture sample (Dreher et al. 2007), and subsampling heavily (Settlage et al. 2008).

**Sex Ratio**

While the sex ratio was approximately even in Pittsburg both years, it was heavily male biased in Milan. The male-bias in Milan was surprising and may indicate either a difference in the demography of that population or relate to behavior. Alternatively, an unequal probability of capture may exist due to behavioral differences between the sexes such as larger male home ranges exposing them to more traps, or trap placement was too sparse for adequate female capture. However, the relative proximity of Pittsburg and Milan suggest different behavioral responses are unlikely.

A more likely explanation is that the male-biased sex ratio is reflective of the population. I further explored this issue by examining the harvest data for towns in the Milan study area (Cambridge, Dummer, Milan, and Millsfield) in 2000-2005 (Table 10). In the 3 consecutive years prior to this study, there was a female biased harvest of 44 females and 30 males (A. Timmins, unpublished data) that may relate to the male-biased sex ratio. Schwartz and Franzmann (1992) maintain that removing females in excess of recruitment will reduce female density and consequently lower sustainable yield.

**Table 10.** Black bear harvest by study area and sex, including the sex ratio of harvested bears from 2000-2005 in New Hampshire (A. Timmins, NH Bear Project Leader, unpublished data).

| Year | Total harvest | M | F | Ratio |
|---|---|---|---|---|
| ***Pittsburg*** | | | | |
| 2000 | 10 | 7 | 3 | 2.33 |
| 2001 | 10 | 6 | 4 | 1.50 |
| 2002 | 7 | 5 | 2 | 2.50 |
| 2003 | 18 | 10 | 8 | 1.25 |
| 2004 | 14 | 8 | 6 | 1.33 |
| 2005 | 11 | 4 | 7 | 0.57 |
| *Total* | | 40 | 30 | |
| ***Milan*** | | | | |
| 2000 | 24 | 11 | 13 | 0.85 |
| 2001 | 19 | 9 | 10 | 0.90 |
| 2002 | 11 | 4 | 7 | 0.57 |
| 2003 | 33 | 15 | 18 | 0.83 |
| 2004 | 18 | 7 | 11 | 0.64 |
| 2005 | 23 | 8 | 15 | 0.53 |
| *Total* | | 54 | 74 | |

Because recruitment of new females into the study area is not immediate, recovery from excess harvest requires many years. Thus, harvest statistics, harvest method, and sex ratio should be closely monitored in Milan to determine whether harvest techniques favor females and result in a skewed sex ratio. The detection of a biased sex ratio is another advantage of the genetic tagging method over the traditional methods used by the NHFG, as the latter method cannot detect this bias.

**Individual Bear Capture Patterns**

Because the trapping season was 8 weeks, many bears were caught in >1 trap. About 50% of males were caught in >1 trap in both study areas, and those bears moved an average of 5.8 km (3.6 mi) between traps in Pittsburg; females moved an average of 3.8 km (2.4 mi). Similarly, males in Milan moved an average of 6.4 km (4 mi) and females 3.8 km (2.4 mi). These results are consistent with our knowledge of bear biology; males generally have larger home ranges and travel farther distances than females (Garshelis and Pelton 1981, Rogers 1987, Koehler and Pierce 2003).

As bears have a high survival rate after 2 years of age (Lee and Vaughan 2005), I expected to capture some of the same individuals in consecutive years. Thirty-seven percent of the genotypes were sampled in both years in Pittsburg (10 males and 14 females), and 28% in Milan (11 males and 8 females). The average probability of being captured during each year of the study was 71% (0.71) in Pittsburg and 60% (0.60) in Milan. Therefore, the expected probability of being captured both years was 50% ($0.71^2$x100) in Pittsburg and 36% ($0.60^2$x100) in Milan. While the observed recapture rate of individuals in both years is less than expected, they are not unreasonable. In fact, more of the sampled genotypes may have been identical, but this was difficult to confirm

due to methodological differences in the 2 years. In the first year alleles were scored by the computer program FLEXI-BIN and in the second year alleles were scored manually to correct shifting patterns found when samples were run multiple times. As a result of this methodological change, some individual genotypes may not have matched perfectly in the 2 years.

To characterize space use by individual bears, I mapped the trap locations for males and females caught in 2006 by constructing polygons using trap sites as points (Fig. 19-22). Figures 20 and 22 illustrate that males have a larger trapping range and Figures 19 and 21 illustrate the clustered spatial distribution of females that are consistent with expectations of female philopatry.

## Population Structure and Spatial Genetic Patterns

### Population Structure

Mobility and dispersal distance are related to population structure as animals with high mobility such as wide-ranging carnivores are expected to have minimal genetic structure (Wayne and Koepfli 1996). Bears are generally solitary and wide-ranging, and with this information alone, I would expect panmictic populations. Related to mobility, however, is social organization, and animals found in structured kin groups display high genetic structure as compared to solitary animals (Double et al. 2005). In bears, the presence of philopatry leads to genetic structure because matrilineal kin groups are found in proximity (Onorato et al. 2004, Moyer et al. 2006). I therefore expected to find population differentiation between Pittsburg and Milan because female philopatry should produce genetic structure. In addition, the study sites were approximately 43 km (27 miles) apart. While males are known to travel such distances, it is unlikely females

**Figure 19**. Polygons depicting all individual female bears (N = 32) captured in 2006 in Pittsburg, NH.


**Figure 20.** Polygons depicting all individual male bears (N = 33) captured in 2006 in Pittsburg, NH.

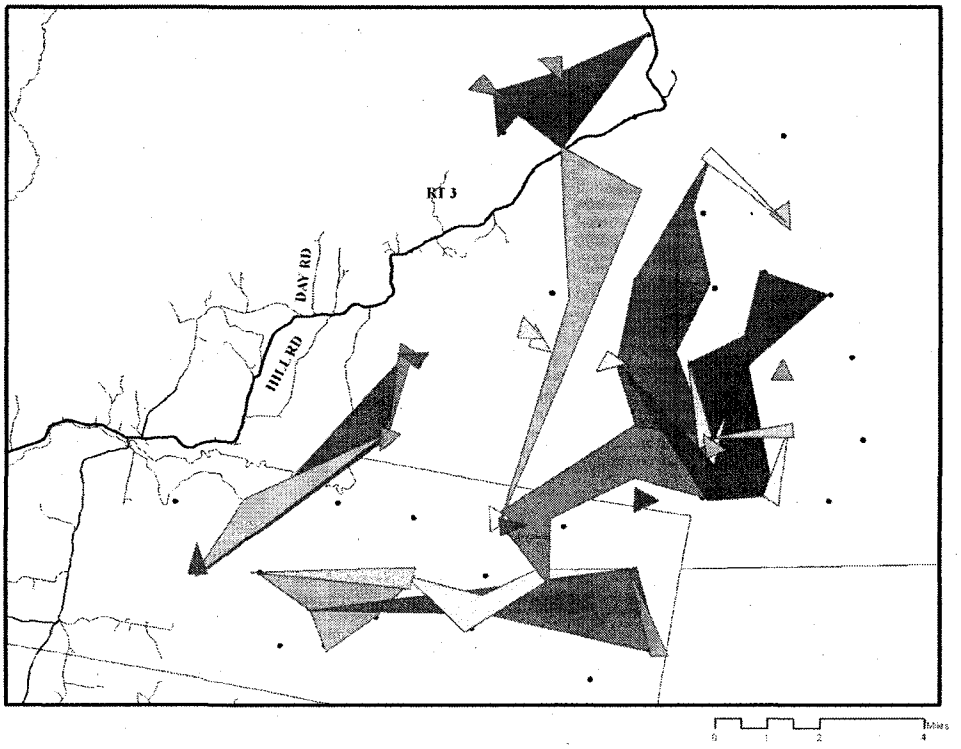


**Figure 21.** Polygons depicting all individual female bears (N = 28) captured in 2006 in Milan, NH.

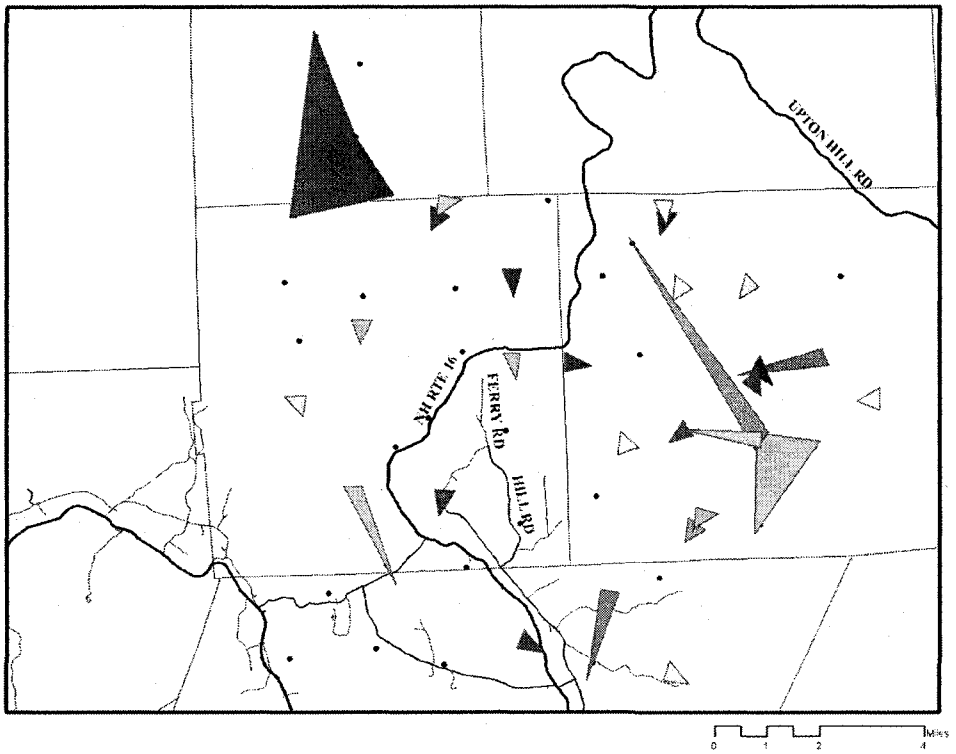
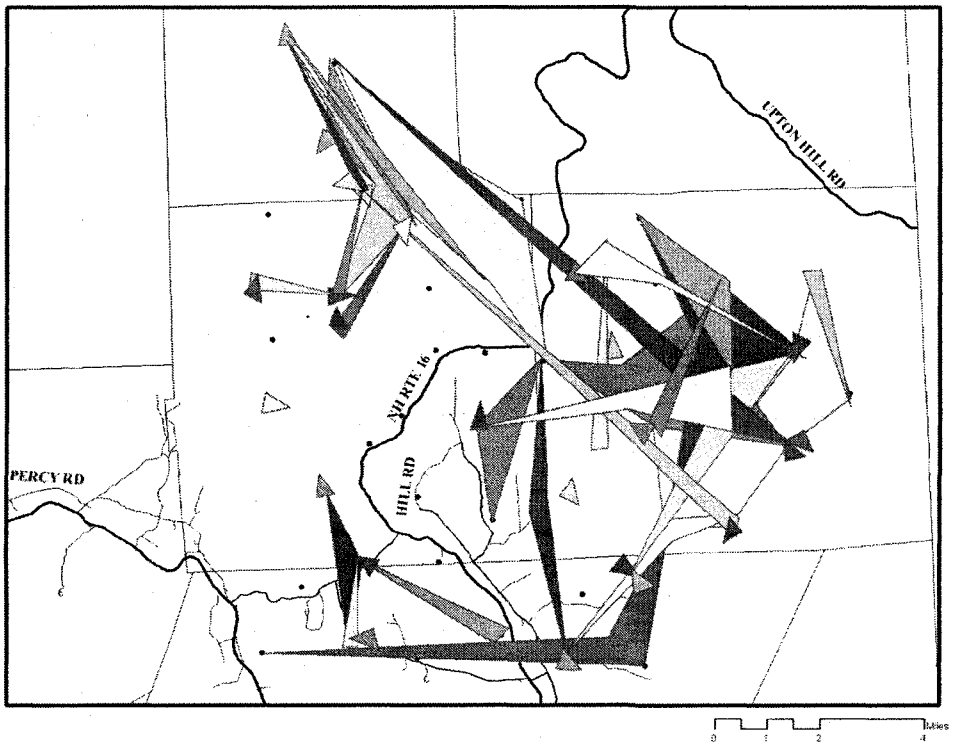



**Figure 22.** Polygons depicting all individual male bears (N = 51) captured in 2006 in Milan, NH.



disperse that far (Rogers 1987). For this reason, I expected the 2 study sites to be distinct populations with the potential for male dispersal. The Fst and relatedness genetic analyses found population differentiation, confirming this expectation, while the program STRUCTURE did not. In the presence of spatial genetic structure and isolation by distance, the program STRUCTURE is not well suited to the data because many individuals may have mixed membership in multiple groups challenging interpretation of the results (Pritchard et al. 2007). Also, the STRUCTURE method is designed to detect subpopulations without prior information, so inputting prior information and generating significant $F_{ST}$ results can be more powerful (Pritchard et al. 2007). I conclude that in this case the $F_{ST}$ and relatedness analyses are more sensitive to the data and Pittsburg and Milan are distinct populations that may occasionally be connected by dispersing males.

**Female Philopatry and Spatial Genetic Structure**

The average relatedness of males and females was equal, and when pairs of individuals at each study site were assessed for relatedness, the majority of dyads were unrelated. Due to female philopatry, I expected females to exhibit a high degree of relatedness. Solitary, carnivorous females are predicted to be philopatric to minimize costs and encourage reproductive success (Waser and Jones 1983). Philopatry has often been used to characterize female space use in black bears (Elowe and Dodge 1989, Schwartz and Franzmann 1992), but only recently has genetic evidence been used to support it (Onorato et al. 2004). Anecdotal evidence to support philopatry in bears initially came from Rogers (1987), who suggested that maternal black bears show tolerance to daughters in their home range beyond the age of independence. However, Schenk et al. (1998) found spatial distribution and patterns of home range overlap were

independent of genetic relatedness. Several weaknesses of the Schenk et al. (1998) study are: 1) they used mitochondrial DNA fingerprinting, which is less sensitive to relatedness patterns than microsatellites; and 2) while the researchers did not find home range overlap, this does not disprove philopatry as relatives can be proximate but not overlap. Using microsatellites, Onorato et al. (2004) found evidence of higher relatedness among female black bears in Texas, supporting the existence of female philopatry. Similarily, Moyer et al. (2006) found evidence of a correlation between spatial proximity and relatedness among individual bears in Florida.

Assuming the presence of female philopatry, I would expect females to be more closely related to each other than males. The Wilcoxin signed-rank test indicated that the mean relatedness values for the 2 sexes were not different (male $r = 0.073$, female $r = 0.071$). This result did not meet expectations based on female philopatry and may be due to a combination of factors. One possible explanation is that female philopatry and relatedness may be difficult to detect in hunted populations. It should be easier to detect the presence of philopatry in a population without harvest pressure because that population would have evolved in natural ecological conditions. In a population with harvest pressure, related females may be taken, making it more difficult to detect high female relatedness or philopatry. Bears are hunted in both Pittsburg and Milan, and this may contribute to the difficulty in detecting high female relatedness. It is also possible that I didn't have the resolution to pick up the relatedness patterns as this study was limited to 6 loci.

While the relatedness analyses did not support the predictions based on female philopatry, the spatial autocorrelation analyses did. These results affirmed my

expectation as spatial genetic structure was detected in females, but not in males. Mechanisms that account for these gender specific spatial patterns corroborate previous research suggesting female philopatry and male-biased dispersal. Spatial structure in the female population through 5 km illustrates that related individuals are found in proximity. This result suggests that female bears in northern New Hampshire establish home ranges on average within 5 km of their mother. In males, low relatedness and a lack of spatial structure in both brown and black bears have been confirmed in other genetic studies (Onorato et al. 2004, Stoen et al. 2005). The lack of spatial structure in males was expected, as males disperse and are presumably not related to other spatially proximate individuals. As no genetic structure was detected through 20 km, this suggests males in northern New Hampshire disperse further than 20 km from their natal territory.

**Landscape Resistance to Gene Flow**

In black and brown bears, roads have been linked to population differentiation (Thompson 2003, Proctor et al. 2005). Using similar genetic tagging methods, Thompson (2003) found US highway 64 created population structure in her North Carolina study area, suggesting that the highway acted as a boundary to gene flow. Proctor et al. (2005) further corroborated this, as they found strong evidence that the presence of a large highway and associated human settlement was fragmenting grizzly bear populations on the US-Canadian border near Alberta, Canada. They also found females were more sensitive to the boundary of the road, and expressed concern that the road was limiting connectivity of the grizzly bear subpopulations in the area.

As roads have been found to enhance population differentiation (Thompson 2003, Proctor et al. 2005), I predicted Route 3 and the Route 16-Androscoggin River corridor

would act as barriers to gene flow in bears at each study site. Results indicated that Route 3 in Pittsburg may not be a significant deterrent, but the Route 16-Androscoggin River corridor might be. In males, there was no genetic differentiation between the subpopulations of bears on either side of Route 3. Rather, in Pittsburg both males and females separated by the road seemed to be structured as a single continuous population. The analysis may have been biased due to sample size, however, as both the male and female eastern subpopulations had 4 times the number of samples when compared to the western subpopulations (Fig. 14-15). According to Brody and Pelton (1989) bears avoid roads in areas open to hunting and are attracted to roads in sanctuaries by the presence of human food. In their study in North Carolina, Brody and Pelton assessed the number of bear crossings on roads of different traffic levels and found that as traffic levels increased, bears' avoidance increased. While Route 3 is a two-lane highway with a speed limit of 55 mph, the vehicle load is light and probably does not greatly inhibit bear movement. It is also important to note that the portion of Route 3 with hair traps on either side was forested up to the road with little human settlement. Therefore, it is not surprising that the road does not function as a barrier to gene flow in this location. The majority of human settlement in Pittsburg is further south around Lake Francis and Back Lake. It is possible that bears may show avoidance of Route 3 in those areas due to increased road traffic and human settlement. Unfortunately, this hypothesis cannot be tested due to lack of traps in that area.

In Milan, the 2 male subpopulations divided by the Route 16-Androscoggin River corridor were not found to be genetically distinct using the relatedness tests. In contrast, the 2 female subpopulations show marginal genetic distinction as the East population (N

= 20) is more related to itself than to the whole. The West showed a lack of genetic distinction, probably due to small sample size (N = 8). In addition to the presence of road and river, these landscape features also have a modest amount of human settlement along them, especially south of Pontook Reservoir, which may deter bear movement. When reviewing the trapping ranges of individual bears as shown by trap visitation, no females visited traps across the river or the adjacent Route 16 (Fig. 21). However, there is evidence that at least 4 males crossed both these barriers because they visited trap sites on both sides (Fig. 22). It is possible the river discourages female movement but not male movement as females have smaller home ranges and the river may act as a natural boundary, especially to maternal bears with cubs. In contrast, males have larger home ranges and could easily traverse a river to visit another part of their home range. While it is clear from male trapping patterns that these landscape features are not barriers to movement, they do influence the spatial genetic structure of bears. It is likely that these physical landscape features are convenient markers for individual bears to distinguish boundaries, and the genetic patterns reflect this structure. Landscape barriers to gene flow can cause conservation concern as they may increase the probability of inbreeding and genetic drift (Thompson 2003) and disrupt immigration or recolonization (Lande 1988). While there is no conservation concern or danger of inbreeding in Milan, it is important to document the potential impact of landscape barriers to aide in future management or conservation. The male-biased sex ratio is of more immediate concern in Milan and warrants further attention.

# CHAPTER 5

## SUMMARY

This study was designed to compare density estimates derived from genetic tagging methods in 2 consecutive years from 2 study sites presumed to have different bear densities, with density estimates derived from hunter-harvest and bear mortality data used by the NHFG. The density estimates generated from the genetic tagging method were consistent in the 2 consecutive years. In 2006, the estimated number of bears in Pittsburg (315 $km^2$) was 70, corresponding to a density range (95% CI) of 0.16-0.28 bears/$km^2$. In 2007, the Pittsburg (400 $km^2$) estimate was similar: 78 bears with an overlapping density range (95% CI) of 0.15-0.24 bears/$km^2$. In Milan (440 $km^2$) during 2006, the estimated number of bears was 106 corresponding to a density range (95% CI) of 0.13-0.35 bears/$km^2$. The 2007 Milan estimate (371 $km^2$) was similar with 99 bears and an overlapping density range (95% CI) of 0.19-0.34 bears/$km^2$. The difference in bear densities may be related to food availability, with increased clear-cutting in Milan providing higher quality habitat and contributing to higher bear densities.

The NHFG density estimates for the north region were similar to the estimates derived from the genetic tagging study. The density estimates for the north region were 0.22-0.25 bears/$km^2$ in 2005-2007 (NHFG Federal Aid Reports 2006, 2007, 2008). The genetic tagging population estimates in Pittsburg were about 9% lower (0.20-0.22 bears/$km^2$) than the NHFG estimates, whereas the Milan estimates were about 13% higher (0.24-0.27 bears/$km^2$). The NHFG density estimate (0.23 bears/$km^2$) fell within

the 95% confidence interval of the genetic tagging estimate in Pittsburg in 2006, but not 2007 (0.25 bears/km$^2$). In Milan the NHFG density estimate fell within the 95% confidence interval in both years (2006: 0.13-0.35 bears/km$^2$; 2007: 0.19-0.34 bears/km$^2$).

Density estimates derived from mortality and hunter observation rates may be reasonable and more cost effective for a regional estimate. The genetic tagging methods were able to detect demographic variation at a local scale, and these methods may improve the regional approach when there are management concerns at a local level. Another advantage to the genetic tagging methods is the ability to ascertain sex-ratios. While the sex ratios at each study site were consistent in the 2 years, the sex ratio was heavily male biased in Milan (2006, 35M:20F; 2007, 39M:20F) and may be a result of excessive female harvest. The biased sex ratio warrants a closer inspection of harvest statistics focusing on harvest method in that area to determine whether harvest techniques favor females and result in a skewed sex ratio.

I used the genetic information to identify population and spatial genetic structure and to see if landscape features such as roads and rivers caused resistance to gene flow. Through consensus, I found that Pittsburg and Milan were genetically distinct ($F_{ST}$ = 0.024, P = 0.05). I also found a positive relationship between genetic and geographic distance (R = 0.13, P>0.0001). Contrary to expectations of female philopatry, relatedness values of males (r = 0.073) and females (r = 0.071) were similar. However, a combination of factors probably led to the lack of detection of high female relatedness. As Pittsburg and Milan are both hunted populations, high female relatedness may be difficult to detect, and this study was also limited by 6 loci. Results of the spatial

analyses show spatial genetic structure in females through 5 km. This result suggests that female bears in northern New Hampshire establish home ranges within 5 km of their mother. The spatial autocorrelation analysis is consistent with a spatial structure organized into female kin groups, as expected from female philopatry. As expected, no genetic spatial structure was detected through 20 km in males, suggesting they disperse further than 20 km from their natal territory. The analysis of landscape resistance to gene flow provided no evidence that Route 3 in Pittsburg restricts gene flow. The Route 16-Androscoggin River corridor in Milan may be a genetic boundary to females, but not males.

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# APPENDICES

**Appendix A.** Black bear population estimates from 10 closed mark-recapture models in program CAPTURE from DNA analysis of hair collected at hair snares during 8 trap sessions in the summer 2006 in Pittsburg, NH. N represents the estimated number of bears in the approximately 387.5 $km^2$ (149.6 $mi^2$) study area. Mh-Jackknife model was selected as the best estimator.

| Model & Estimator | N | SE | 95% CI |
|---|---|---|---|
| Mo-Null | 71 | 2.3 | 69-78 |
| Mt-Darroch | 70 | 2.1 | 69-77 |
| Mb-Zippin | 89 | 14.6 | 74-139 |
| **Mh-Jackknife** | **107** | **13.9** | **88-144** |
| Mh-Chao | 104 | 17.8 | 83-157 |
| Mbh-Removal | 89 | 14.6 | 74-139 |
| Mbh-Pollock | 109 | 18.3 | 86-162 |
| Mth-Chao | 106 | 14.8 | 86-147 |
| Mtb-Burnham | 150 | 393.4 | 70-2765 |
| Mt-Chao | 96 | 14.1 | 79-138 |

**Appendix B.** Black bear population estimates from 10 closed mark-recapture models in program CAPTURE from DNA analysis of hair collected at hair snares during 5 trap sessions in the summer 2006 in Pittsburg, NH. N represents the estimated number of bears in the approximately 315.3 $km^2$ (122.7 $mi^2$) study area. Mbh-Pollock was selected as the best estimator.

| Model & Estimator | N | SE | 95% CI |
|---|---|---|---|
| Mo-Null | 54 | 2.5 | 52-62 |
| Mt-Darroch | 54 | 2.2 | 52-61 |
| Mb-Zippin | 71 | 16.7 | 56-133 |
| Mh-Jackknife | 67 | 7.4 | 58-88 |
| Mh-Chao | 65 | 9.0 | 56-94 |
| Mbh-Removal | 71 | 16.7 | 56-133 |
| **Mbh-Pollock** | **70** | **10.0** | **58-100** |
| Mth-Chao | 67 | 8.5 | 57-92 |
| Mtb-Burnham | 87 | 105.5 | 53-731 |
| Mt-Chao | 61 | 6.6 | 54-82 |

**Appendix C.** Black bear population estimates from 10 closed mark-recapture models in program CAPTURE from DNA analysis of hair collected at hair snares during 6 trap sessions in the summer 2007 in Pittsburg NH. N represents the estimated number of bears in the approximately 406.6 $km^2$ (157.0 $mi^2$) study area. Mh-Jackknife was selected as the best estimator.

| Model & Estimator | N | SE | 95% CI |
|---|---|---|---|
| Mo-Null | 69 | 2.5 | 67-72 |
| Mt-Darroch | 69 | 2.2 | 67-71 |
| Mb-Zippin | 78 | 8.8 | 69-87 |
| **Mh-Jackknife** | **83** | **7.1** | **76-90** |
| Mh-Chao | 80 | 8.0 | 72-88 |
| Mbh-Removal | 98 | 34.7 | 63-132 |
| Mbh-Pollock | 100 | 14.5 | 86-115 |
| Mth-Chao | 81 | 7.6 | 73-89 |
| Mtb-Burnham | 107 | 76.5 | 31-184 |
| Mt-Chao | 76 | 6.2 | 70-82 |

**Appendix D.** Black bear population estimates from 10 closed mark-recapture models in program CAPTURE from DNA analysis of hair collected at hair snares during 5 trap sessions in the summer 2007 in Pittsburg NH. N represents the estimated number of bears in the approximately 399.9 $km^2$ (154.4 $mi^2$) study area. Mh-Jackknife was selected as the best estimator.

| Model & Estimator | N | SE | 95% CI |
|---|---|---|---|
| Mo-Null | 62 | 2.6 | 60-70 |
| Mt-Darroch | 62 | 2.2 | 60-69 |
| Mb-Zippin | 68 | 7.4 | 61-94 |
| **Mh-Jackknife** | **78** | **8.3** | **67-101** |
| Mh-Chao | 75 | 9.6 | 65-105 |
| Mbh-Removal | 81 | 25.3 | 62-190 |
| Mbh-Pollock | 90 | 12.7 | 74-125 |
| Mth-Chao | 77 | 8.9 | 67-103 |
| Mtb-Burnham | 76 | 23.4 | 61-183 |
| Mt-Chao | 70 | 6.9 | 63-92 |

**Appendix E.** Black bear population estimates from 10 closed mark-recapture models in program CAPTURE from DNA analysis of hair collected at hair snares during 8 trap sessions in the summer 2006 in the Milan extended area of NH. N represents the estimated number of bears in the approximately 439.8 $km^2$ (169.8 $mi^2$) study area. Mbh-Pollock was selected as the best estimator.

| Model & Estimator | N | SE | 95% CI |
|---|---|---|---|
| Mo-Null | 100 | 6.4 | 91-116 |
| Mt-Darroch | 99 | 6.1 | 91-115 |
| Mb-Zippin | 252 | 209.5 | 108-1197 |
| Mh-Jackknife | 147 | 19.1 | 119-195 |
| Mh-Chao | 134 | 21.3 | 107-194 |
| Mbh-Removal | 252 | 209.5 | 108-1197 |
| **Mbh-Pollock** | **123** | **18.3** | **100-176** |
| Mth-Chao | 114 | 23.7 | 114-210 |
| Mtb-Burnham | 123 | 52.4 | 88-361 |
| Mt-Chao | 124 | 17.5 | 102-173 |

**Appendix F.** Black bear population estimates from 10 closed mark-recapture models in program CAPTURE from DNA analysis of hair collected at hair snares during 5 trap sessions in the summer 2006 in the Milan extended area of NH. N represents the estimated number of bears in the approximately 439.8 $km^2$ (169.8 $mi^2$) study area. Mth-Chao was selected as the best estimator.

| Model & Estimator | N | SE | 95% CI |
|---|---|---|---|
| Mo-Null | 84 | 10.6 | 70-113 |
| Mt-Darroch | 81 | 9.6 | 69-107 |
| Mb-Zippin | N/A | N/A | N/A |
| Mh-Jackknife | 108 | 13.9 | 87-143 |
| Mh-Chao | 102 | 20.2 | 76-160 |
| Mbh-Removal | 96 | 50.1 | 62-324 |
| Mbh-Pollock | 92 | 13.4 | 74-128 |
| **Mth-Chao** | **106** | **25.0** | **76-182** |
| Mtb-Burnham | 110 | 108.9 | 61-2200 |
| Mt-Chao | 88 | 14.5 | 70-130 |

**Appendix G.** Black bear population estimates from 10 closed mark-recapture models in program CAPTURE from DNA analysis of hair collected at hair snares during 6 trap sessions in the summer 2007 in the Milan extended area of NH. N represents the estimated number of bears in the approximately 397.8 $km^2$ (153.6 $mi^2$) study area. Mbh-Pollock was selected as the best estimator.

| Model & Estimator | N | SE | 95% CI |
|---|---|---|---|
| Mo-Null | 79 | 4.6 | 73-92 |
| Mt-Darroch | 78 | 4.4 | 73-90 |
| Mb-Zippin | 149 | 77.5 | 85-461 |
| Mh-Jackknife | 100 | 11.1 | 85-129 |
| Mh-Chao | 94 | 11.8 | 80-128 |
| Mbh-Removal | 149 | 77.5 | 85-461 |
| **Mbh-Pollock** | **113** | **16.4** | **91-158** |
| Mth-Chao | 98 | 13.4 | 82-137 |
| Mtb-Burnham | 200 | 672.8 | 72-4699 |
| Mt-Chao | 88 | 9.2 | 77-115 |

**Appendix H.** Black bear population estimates from 10 closed mark-recapture models in program CAPTURE from DNA analysis of hair collected at hair snares during 5 trap sessions in the summer 2007 in the Milan extended area of NH. N represents the estimated number of bears in the approximately 371.2 $km^2$ (143.3 $mi^2$) study area. Mbh-Pollock was selected as the best estimator.

| Model & Estimator | N | SE | 95% CI |
|---|---|---|---|
| Mo-Null | 70 | 4.8 | 64-83 |
| Mt-Darroch | 69 | 4.5 | 64-82 |
| Mb-Zippin | 142 | 95.2 | 73-558 |
| Mh-Jackknife | 94 | 11.5 | 78-124 |
| Mh-Chao | 89 | 14.2 | 72-131 |
| Mbh-Removal | 142 | 95.1 | 73-557 |
| **Mbh-Pollock** | **99** | **14.1** | **80-137** |
| Mth-Chao | 90 | 14.5 | 73-133 |
| Mtb-Burnham | 143 | 285.2 | 63-1953 |
| Mt-Chao | 80 | 10.3 | 68-111 |

**Appendix I.** Institutional Care and Use Committee Approval Form

UNIVERSITY of NEW HAMPSHIRE

March 27, 2006

Peter Pekins
Natural Resources, James Hall
Durham, NH 03824

**IACUC #:** 060301
**Approval Date:** 03/24/2006
**Review Level:** B
**Project:** Black Bear Abundance in Northern New Hampshire

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category B on Page 4 of the Application for Review of Vertebrate Animal Use in Research or Instruction - *the study involves either no pain or potentially involves momentary, slight pain, discomfort or stress.* The IACUC made the following comments on this protocol:

1. *In the future, the investigator should consider limiting the summary of proposed animal use (Section III, A).*

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

**Please Note:**
1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

If you have any questions, please contact either Roger Wells at 862-2726 or Julie Simpson at 862-2003.

For the IACUC,

Jessica A. Bolker, Ph.D.
Chair

cc: File

**Research Conduct and Compliance Services, Office of Sponsored Research, Service Building, 51 College Road, Durham, NH 03824-3585 * Fax: 603-862-3564**